



10 August 2012 \$16

Science

WORKING WITH WASTE

AAAS

SPECIAL SECTION

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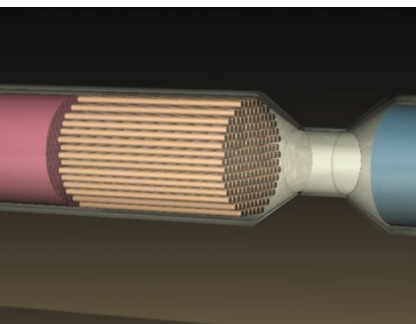
COVER

Recycling aluminum cans (shown here in bales at a facility in Philadelphia, PA) is just one of the many approaches directed toward doing something productive with the world's ever-expanding stream of waste. In the special section Working with Waste (page 662), we survey multifaceted efforts to tackle this global challenge. For the story behind the cover, go to <http://scim.ag/cov6095>.

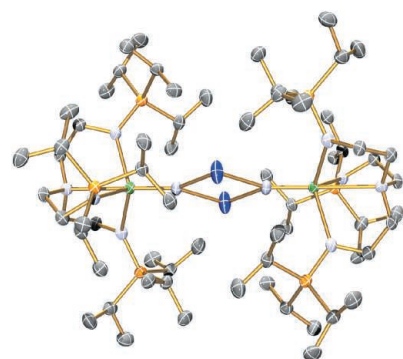
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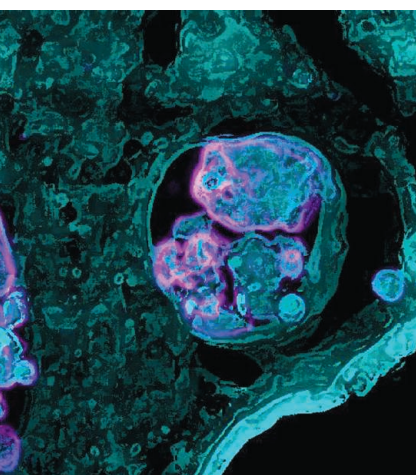
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Full text at www.sciencemag.org/cgi/content/full/337/6095/646-b

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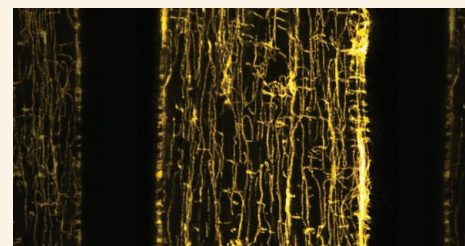
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RESEARCH ARTICLE: A Combination of Buprenorphine and Naltrexone Blocks Compulsive Cocaine Intake in Rodents Without Producing Dependence

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SCIENCE CAREERS

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Taken for Granted: Crossing the Rubicon

B. L. Benderly

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http://scim.ag/TFG_Rubicon

Perspective: Science Training and Mental Health

M. P. DeWhyse

In the wake of the Colorado shootings, the scientific community should pay more attention to the psychological well-being of emerging scientists.

http://scim.ag/Training_MentalHealth

Gambling on Transformative Research

E. Pain

Scientists with the right skills and attitude find limited but increasing opportunities to pursue high-risk, high-reward research.

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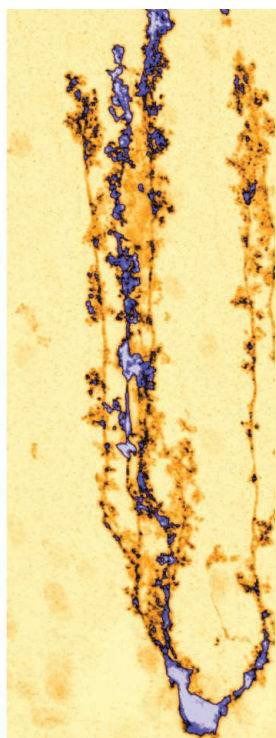
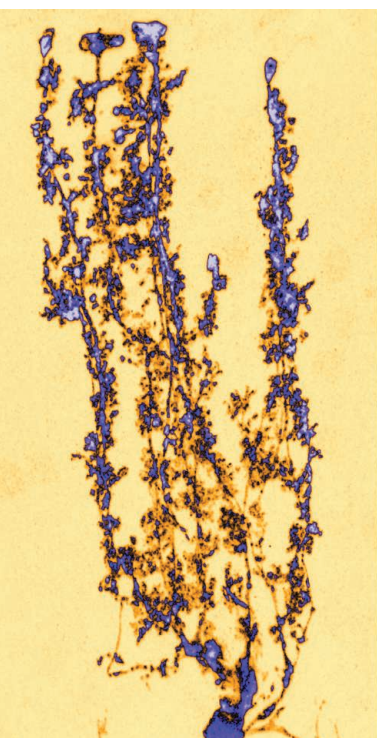
On the 10 August Science Podcast: Garbology 101, challenges in metal recycling, the "yuck factor," and an end to waste.

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ADVANCING SCIENCE. SERVING SOCIETY



Crucial Cerebellar Glial Cells

The role of glial cells and their interaction with neurons in normal behavior is unclear. To address this question, **Saab *et al.*** (p. 749, published online 5 July) studied a special type of glial cell in the cerebellum. Conditional mutant mice were produced in which the two glutamate receptor subunits normally present in Bergmann glial cells were efficiently ablated in a temporally controlled manner. Glutamate signaling of the glial cells contributed to the structural and functional integrity of the cerebellar network. Bergmann glial cells also played a role in the “fine-tuning” of neuronal processing, which is crucial for the fast and precise control of complex motor behavior.

Lattice Trailing the Electrons

Superconducting order recedes with decreased chemical doping in a typical iron-based superconductor family. Thus, the symmetry of the material is broken by almost simultaneous antiferromagnetic and structural phase transitions. However, some pnictides also exhibit an electronic nematic transition manifested by anisotropy of the electrical resistance. Because this anisotropy occurs at the same time as the structural transition, it is not clear whether it is a consequence of the broken crystal lattice symmetry or its cause. **Chu *et al.*** (p. 710) performed constant strain experiments on the series $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$, which can distinguish between the two scenarios, and confirm that the electrons drive the lattice transition.

UN Coordination

Uranium is best known for its radioactivity. From the standpoint of lower-energy chemistry, uranium is also intriguing for its bonding motifs, which involve trinodal f orbitals. **King *et al.*** (p. 717, published online 28 June; see the Perspective by **Sattelberger and Johnson**) synthesized and isolated a molecule bearing a uranium-nitrogen triple bond. Theoretical calculations allowed the mapping of the orbital interactions, distinguishing it from similar motifs in compounds of lighter metals. The preparation required use of a rigid,

bulky ligand framework to keep the reactive uranium nitride group from binding to another molecule nearby, a pathway that has plagued prior attempts to prepare this class of compounds.

Constraining the Birthplace of Asteroids

Many primitive meteorites originating from the asteroid belt once contained abundant water that is now stored as OH in hydrated minerals. **Alexander *et al.*** (p. 721, published online 12 July) estimated the hydrogen isotopic compositions in 86 samples of primitive meteorites that fell in Antarctica and compared the results to those of comets and Saturn’s moon, Enceladus. Water in primitive meteorites was less deuterium-rich than that in comets and Enceladus, implying that, in contradiction to recent models of the dynamical evolution of the solar system, the parent bodies of primitive meteorites cannot have formed in the same region as comets. The results also suggest that comets were not the principal source of Earth’s water.

Earthquake in a Maze

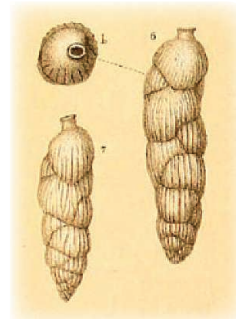
The 11 April 2012 magnitude 8.6 earthquake offshore of Sumatra was the largest measured earthquake along a strike-slip boundary that modern seismological instruments have ever recorded. Despite its size and proximity to a large

population, there was no subsequent tsunami and there were no reported fatalities. **Meng *et al.*** (p. 724, published online 19 July) used teleseismic data from seismological networks in Japan and Europe to image the source of high-frequency radiation generated by the earthquake to understand the mechanics of this unique event. The resultant back projections showed that the earthquake slowly ruptured along a complex series of faults. The deeper-than-usual rupture path and large stress drop are both features that may not be unique to this earthquake, suggesting that regions in a similar tectonic environment may have the potential for more complex—or larger—intraplate earthquakes than might have been expected.

Cycling Down

The Mid-Pleistocene Transition, which lasted from approximately 1.25 million to 700 thousand years ago, was a period during which the dominant periodicity of Earth’s climate cycles inexplicably changed from 41 thousand to 100 thousand years.

This change is clearly apparent in the oxygen isotopic composition of many calcifying marine organisms, but changes in both ice volume and temperature affect the signal, and so exactly what the signal means has remained unclear. **Elderfield *et al.*** (p. 704; see the Perspective by **Clark**) separated these two effects by measuring both the oxygen isotopic makeup and the Mg/Ca (a proxy that reflects changes in temperature only) of certain benthic foraminifera. The findings reveal the contributions of ice volume and temperature to glacial cycles, suggest when and why the Mid-Pleistocene Climate Transition occurred, and clarify how carbon is lost from the ocean-atmosphere during deglaciations but also changes because of ocean circulation.



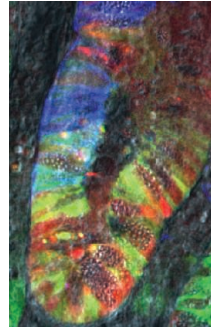
Phosphoinositide Contributions

To study the roles of phosphoinositides in the plasma membrane of mammalian cells, **Hammond *et al.*** (p. 727, published online 21 June; see the Perspective by **Fairn and Grinstein**) engineered phosphatase molecules that could be targeted to the membrane on demand, where they would alter the concentrations of the phos-

pholipids phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P₂] and phosphatidylinositol 4-phosphate (PI4P). PI4P was thought to provide a major source for the synthesis of PI(4,5)P₂, but depletion of PI4P did not have much effect on synthesis of PI(4,5)P₂. Instead, PI4P appears to help to establish the negative charge at the membrane and thus promote electrostatic interactions with positively charged amino acids in membrane-associated proteins and influencing function of ion channels.

Cancer Stem Cells in Color

One of the liveliest debates in contemporary cancer research centers on whether cancer stem cells (CSCs) exist and, if so, how these cells are defined phenotypically. CSCs are hypothesized to be a small population of cells within a tumor that are endowed with the unique capacity to drive tumor growth—a scenario that in principle would offer important therapeutic opportunities. By studying mice expressing multicolor reporter genes, **Schepers *et al.*** (p. 730, published online 1 August) were able to visualize and monitor the fate of a candidate stem cell for intestinal adenomas, an early stage of cancer. This “lineage tracing” analysis suggests that tumor cells expressing the intestinal crypt stem cell marker *Lgr5* (leucine-rich repeat containing G protein-coupled receptor 5) are the cells that fuel the growth of intestinal adenomas.



Current on Demand

Deep brain electrical stimulation can be a successful therapy in Parkinson's disease, in depression, and in several other psychiatric diseases, especially in drug-resistant cases. Unfortunately, chronic, continuous stimulation is associated with multiple side effects. This could be alleviated by delivering the electrical perturbation only when it is necessary, using closed-loop stimulation. Such an approach is essential for epilepsy, where seizures occur very rarely but with serious consequences. In a rat model for epilepsy, **Berényi *et al.*** (p. 735) prevented seizures by transcranial electrical stimulation using a closed-loop system. Transcranial electrical stimulation was highly effective and reduced seizure duration, on average, by 60 %.

Bio-Inspired Drug Delivery

Noting that platelets naturally migrate to narrowed blood vessels characterized by high fluid shear stress, **Korin *et al.*** (p. 738, published online 5 July; see the Perspective by **Lavik and Ustin**) developed a nanoparticle-based therapeutic that uses a similar targeting mechanism to deliver a drug to vessels obstructed by blood clots. Aggregates of nanoparticles coated with the clot-dissolving drug tPA (tissue plasminogen activator) were designed to fall apart and release the drug only when encountering high fluid shear stress. In preclinical models, the bio-inspired therapeutic dissolved clots and restored normal blood flow at lower doses than free tPA, suggesting that this localized delivery system may help reduce the risk of side effects such as excessive bleeding.

Promoting the Male X Chromosome

In mammals and fruit flies, females have a double dose of the X chromosome compared to males, and to compensate for this imbalance, in fruit flies, transcription from across most of the male X chromosome is boosted by twofold. **Conrad *et al.*** (p. 742, published online 19 July) measured the binding of RNA polymerase II, responsible for the majority of the transcription on the X chromosome, and found a consistent increase at the promoters of genes on the male X chromosome. Thus, the increase in transcription on the male X chromosome is not driven by increased rates of transcriptional elongation, as has been suggested previously, but must involve up-regulation of transcription initiation.

The Conductor in the Thalamus

The pulvinar is the largest thalamic nucleus in the brain but its functions remain unclear. The pulvinar is ideally positioned to synchronize activity across the visual cortex. **Saalmann *et al.*** (p. 753) combined diffusion tensor imaging with multi-electrode recordings from three different brain areas in monkeys to probe thalamo-cortical interactions during visual attention. The pulvinar was found to play a vital role in attention by routing behaviorally relevant information across the visual cortex.

CREDIT: SCHEPERS ET AL.



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An End to Waste?

EVEN THOUGH WASTE IS AN INEVITABLE ACCOMPANIMENT TO ALL PROCESSES, CONTEMPLATING an end to waste can force us to think about how we define, generate, and manage it. Two common definitions of waste are a substance or object that is discarded and the avoidable loss of a resource. The current waste management practices in industrialized countries are widely recognized to be unsustainable, yet it is clear that we are not changing our practices effectively or fast enough. What are the impediments and how can we overcome them?

Current waste management practices include the options of disposal, recovery, recycling, reuse, minimization, and prevention. Reuse begins to blur the definition of waste: If an unwanted by-product of one industry can be used as a feedstock for another, is it a waste or a resource? For example, food and crop waste has been identified as a valuable feedstock for biofuel production. And with improved extraction technologies, wastes generated by past mining activities can serve as a valuable source of mineral resources. More effort is needed to identify such potential opportunities, develop the technologies needed to exploit them, and remove any regulatory or legal constraints to implementation. Such efforts should target the sectors that generate the most waste. In the 27 European Union (EU) member countries, just three sectors—construction and demolition, mining, and manufacturing—generated nearly 74% of all waste in 2008.* One advance is the “end of waste” status developed by the EU Environment Agency, which allows the processed material, no longer classified as waste, to be used in the same way as comparable virgin materials or products. Such end-of-waste regulations were set for scrap metal in March 2011.‡

Under current practices, many environmental costs of production, including waste generation, are externalized; that is, they are not incorporated into the cost of products. For example, the requirement for manufacturers to take back packaging or even products at their end of life can be a critical step in internalizing costs, shifting waste management from disposal to recycling or reuse. But political processes, such as regulation, are generally necessary to achieve such goals. It is therefore critical to raise the awareness of waste as an important societal issue. Even though household waste constituted only 8.5% of the waste generated in the EU in 2008, it is disproportionately important both because a focus on it creates broader social awareness and because higher-value goods such as electronics are being discarded.

Online

sciencemag.org



Podcast interview with author Janet G. Hering (http://scim.ag/ed_6095).

Political aspects are also central to the inefficient use of resources. Agriculture in the arid southwestern United States is often used as an example of the inefficient use of water. This is partly because water rights require beneficial use of the resource (“use it or lose it”), which acts as a perverse incentive to discourage water conservation and must be addressed by changing the legal framework. This can only be achieved through political processes, yet water conservation will also require technological improvements in irrigation and an increased scientific understanding of hydrological and plant growth processes.

Public awareness will be crucial if waste management is to be addressed from the perspective of consumption as well as production. This is essential if a displacement of costs, either in space or in time, is not to be mistaken for a gain in efficiency. For example, production may appear efficient if it need not accommodate later recycling, but those costs are merely shifted (both in time and often in space) from the producer to the recycler. Even real increases in efficiency will ultimately reach a limit because of the fundamental linkage of waste to throughput. Thus, the question of waste also requires that we examine our patterns of production and consumption and adjust them to the inevitable limits of our planetary ecosystem.

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— Janet G. Hering

10.1126/science.1227092

*http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Waste_statistics.

‡www.environment-agency.gov.uk/static/documents/Business/End_of_waste_reg.pdf.



EVOLUTION

Redox Dragonflies

Animals exhibit beautiful and varied color patterns that assist in species and gender recognition and avoiding detection. Sexual maturity is also signaled by color change in insects, birds, and mammals. In dragonflies of the genera *Crocothemis* and *Sympetrum*, the male displays yellow body coloration when young but is bright red when sexually mature. Futahashi *et al.* reveal that the redox state of epidermal ommochrome pigments confers this sex-related change. When the ratio of the reduced form to the oxidized form is higher, as in adult males, red color results. Females and immature males, which are yellow, have a lower ratio. When reductant or oxidant was added to extracted dragonfly pigments *in vitro*, color transitions were observed. In addition, reductant injected into immature males and mature females resulted in a change to the vivid red seen in adult males. These results extend our knowledge of the role of oxidants and reductants in regulating animal pigmentation, as previously shown in fruit flies and butterflies. — BAP

Proc. Natl. Acad. Sci. U.S.A. **109**, 10.1073/pnas.1207114109 (2012).



GENETICS

Genetic Transfer Sighted

In the early 1950s, Martha Chase and Alfred Hershey used radioisotope labeling of the protein and DNA of a bacteriophage to show that DNA, rather than the proteins that they expected, facilitated the transfer of genetic information for new phage replication within a bacterial cell. Van Valen *et al.* now report visual confirmation, in real time, of single fluorescently labeled molecules of DNA from bacteriophage lambda being ejected into live *Escherichia coli* cells. The authors' method reveals properties of the DNA ejection event that differ from those measured by *in vitro* detection of DNA ejected from phage artificially treated with a bacterial outer membrane protein. Transfer to the live cell took several minutes—10 to 100 times longer than had been observed *in vitro*. Further characterization of variable timing of the transfer, pausing during transfer, and rates



of transfer in relation to the amount of DNA transferred begin to shed light on fundamental aspects of the process that remain mysterious. Specifically, they increase our understanding of the relative extent to which transfer is promoted by molecular motors, by the energy of compaction of the DNA in the bacteriophage capsid, or by events within the bacterial cell. — LBR

Curr. Biol. **22**, 1339 (2012).

BIOENGINEERING

Silk Replaces Refrigeration

Both vaccines and antibiotics lessen the burden of disease—one before the fact and one after. Successful treatment often requires a “cold chain,” in which the vaccine or antibiotic must be refrigerated from production to patient. Failure to sustain the cold chain results in loss of therapeutic efficacy, but maintaining it can be expensive, especially in developing countries. Zhang *et al.* demonstrate the use of silk to stabilize labile vaccine compounds at ambient temperatures. Silk fibroin, the protein polymer from silkworm cocoons, can be formed into more than scarves and ties. Specifically, as a film, silk contains tiny pockets that can harbor and protect sensitive molecules. The authors tested the efficacy of silk encapsulation on live measles, mumps, and rubella (MMR) vaccines, as well as the antibiotics penicillin and tetracycline. Their results suggest that such encapsulation can protect the compounds from degradation and

temperature-induced denaturation. The activity of the MMR vaccine and the two antibiotics, as assessed with *in vitro* tests, was even protected in temperatures up to 60°C. This surprising application for silk has the potential to reduce reliance on the cold chain, a possibility that could be especially valuable where electricity and refrigeration are hard to come by. — PJH

Proc. Natl. Acad. Sci. U.S.A. **109**, 10.1073/pnas.1206210109 (2012).

BIOMEDICINE

RAC and Ruin in Melanoma

Despite the increased use of sunscreens, the incidence of melanoma, the most lethal form of skin cancer, remains high. Tumor genome sequencing has led to new therapies targeting BRAF, a protein kinase that is activated by mutation in about 50% of melanomas and helps drive tumor growth. Because the development of resistance to BRAF inhibitors limits their long-term efficacy, there is considerable interest in identifying additional driver mutations that might form the basis of new or combination therapies.

Toward this end, Krauthammer *et al.* sequenced the protein-coding regions of 147 human melanoma genomes. Notably, 9% of sun-exposed melanomas harbored a point mutation in *RAC1*, which encodes a small GTPase (an enzyme hydrolyzing guanosine triphosphate) that regulates cytoskeletal rearrangements. Structural and functional

analysis revealed that the mutation increases RAC1 binding to its downstream effectors, including PAK1 (p21-activated protein kinase), and induces melanocyte growth and migration. PAK kinases are therefore potentially druggable targets for melanoma treatment. In independent work, Hodis *et al.* found the same activating RAC1 mutation in 5% of their melanoma samples. — PAK

Nat. Genet. 10.1038/ng2359 (2012);
Cell 150, 251 (2012).

CHEMISTRY

Aromatizing Ethers

Aryl ethers are common motifs in the production of commercially important organic compounds. Generally, they are prepared from precursors that already contain the aromatic ring (either by coupling a carbon center to a phenolic oxygen or coupling an alcohol to an aryl carbon). Simon *et al.* have developed a complementary approach that combines an alcohol with a cyclohexenone derivative and then aromatizes the framework after carbon-oxygen bond formation. They initially used cupric chloride as a coupling agent, together with O₂ as an oxidant. Adding an organic radical source as co-catalyst and replacing the counterions with triflate together with potassium iodide reduced the necessary copper loading to 10%. The reaction tolerates a range of functional groups, including esters, alkynes (both internal and terminal), and aryl iodides poised for further elaboration by cross-coupling techniques. Control experiments preclude the intermediacy of phenol, thus implicating a mechanism in which alcohol addition to the carbonyl precedes hydrogen abstractions that produce the aromatic ring. — JSY

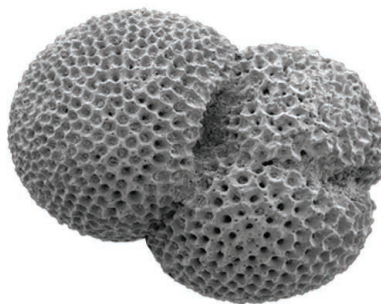
Angew. Chem. Int. Ed. 51, 7537 (2012).

CLIMATE SCIENCE

Understanding Past CO₂

Overall, atmospheric CO₂ levels have decreased during the Cenozoic (the geological era that started 65 million years ago), coinciding with a broad cooling of Earth. Several prominent warm excursions, however, provide opportunities to calibrate the climate of CO₂ and offer comparisons for understanding the long-term effect of human CO₂ emissions. A requirement for such an analysis is a good understanding of past CO₂ levels. One prominent warmer excursion occurred during the Miocene, about 15 million years ago, when temperatures are thought to have warmed by about 3°C and sea level rose as, it is thought, the growth of the Antarctic Ice Sheet abated. Foster *et al.* analyzed two deep-

sea cores and reconstructed detailed CO₂ levels across this excursion based on boron isotopes in foraminifera, a phylum of amoeboid protists commonly associated with marine plankton. The



boron isotopic ratio of seawater varies with pH, which in turn is influenced by atmospheric CO₂ levels. Their data, broadly consistent with other measurements, imply that CO₂ levels during the warmest part of the Miocene excursion were about 400 ppm, comparable to the levels that we have reached recently. Broad excursions in the global ice volume during the Miocene coupled with these modest atmospheric CO₂ levels suggest that the Antarctic Ice Sheet was rather sensitive to this modest forcing at that time. — BH

Earth Planet. Sci. Lett. 341–344, 243 (2012).

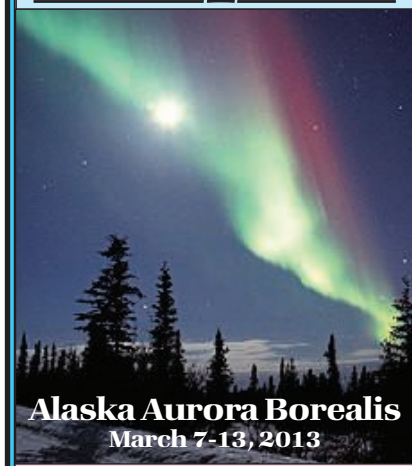
APPLIED PHYSICS

Semiconductor Standards

Modern technology is dependent on components that perform as specified and often within extremely high tolerance. The specification of each component, whether it delivers a current, a voltage, or a resistance, is typically calibrated (although usually not directly) against a quantum standard that is dependent on only fundamental constants. These quantum standards are usually operated at a national laboratory. Secondary standards are then shipped out to factories where the components can be calibrated. Usually, each quantum standard is a separate device: superconductors for voltage and semiconductors for current and resistance. Hohls *et al.* demonstrate an integrated quantized circuit with which the so-called metrological triangle (V, I, and R) can be closed with a single semiconductor-based device. Using GaAs, they combined an integrated single-charge pump with a quantum Hall resistor to provide a quantized voltage source. Such a monolithic approach should be applicable to other materials, such as silicon or graphene, and could provide a platform for developing quantum standard devices on a single chip. — ISO

Phys. Rev. Lett. 109, 056802 (2012).

AAAS Travels

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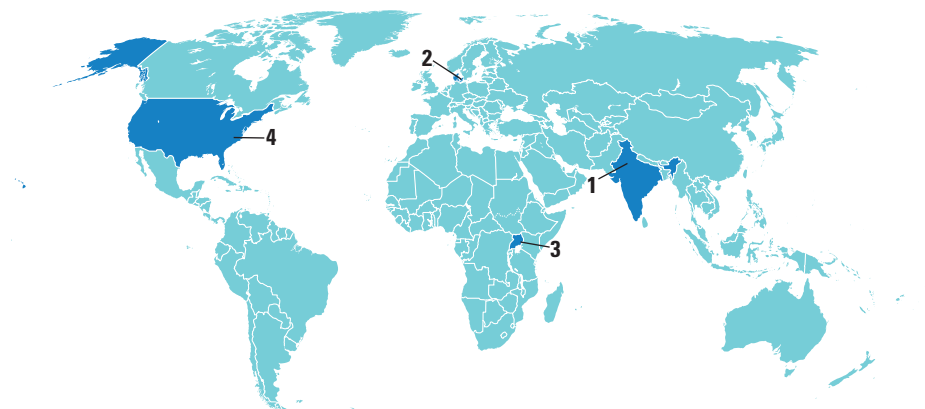
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AROUND THE WORLD



New Delhi 1

India Prepares Orbiting Mars Satellite

India plans to send a small, crewless satellite to orbit Mars in what would be its first visit to the planet.

On 3 August, the Indian cabinet cleared a proposal from the Indian Space Research Organisation (ISRO) for a launch in November 2013. The agency will use its Polar Satellite Launch Vehicle, the same rocket that sent Chandrayaan-1 on a successful mission around the moon in 2008. The government has already allocated about \$41 million for the Mars mission, which will cost an estimated \$112 million.

The satellite will carry up to 25 kg of scientific instruments and track a highly elliptical orbit—500 km by 80,000 km—around the Red Planet. Although details are not available, ISRO officials said the mission's goal is to remotely assess "climate, geology, and the origin, evolution, and sustainability of life on the planet."

No word on whether there will be opportunities for international collaboration. Chandrayaan-1 carried instruments from NASA, the European Space Agency, and Bulgaria.

Copenhagen 2

Squabble Over *NEJM* Paper Puts Spotlight on Antishock Drug

A seemingly small mistake in a paper in *The New England Journal of Medicine* (*NEJM*) landed a Danish physician-researcher in hot water last month after a German company

threatened to sue him. The researcher, Anders Perner of Copenhagen University Hospital, corrected the error—but the episode sheds light on a widespread therapy that some scientists say may do more harm than good.

On 27 June, Perner published a clinical trial in *NEJM* that compared the effects of hydroxyethyl starch (HES), a synthetic derivative of starch that has been used globally for decades to treat sepsis, with treatment by an alternative called Ringer's acetate. The results were not good for HES: After 90 days, 201 of 398 patients in the HES group had died, compared with 172 of 400 patients in Ringer's acetate group.

Perner and *NEJM* amended the original paper after German pharma company Fresenius Kabi threatened legal action, stating that Perner had misidentified the HES compound used in the study as Fresenius's product.

But Perner says the data from his study are likely to apply to Fresenius's drug as well. He contends that the safety of HES compounds—first developed by Fresenius in 1974—has never been adequately assessed by modern standards. <http://scim.ag/HESfight>

Uganda 3

Sixteen Suspected Dead From Ebola

Ugandan health authorities are battling an outbreak of Ebola, an often fatal viral disease. On 6 August, the Ugandan government reported 59 suspected cases, including 16 deaths—but only 10 cases had been confirmed by tests at the Uganda Virus Research Institute in Entebbe. The outbreak started in early July in the western District of Kibaale, where the first cases may have been mistaken for cholera.

There are no drugs or vaccines that pro-



Hot zone. CDC researchers in Entebbe, Uganda, study the recent Ebola outbreak.

tect against Ebola, which is spread through direct contact with patients before or after they die. Outbreaks are usually stamped out in a few weeks by aggressively tracing patients' contacts and isolating them for 3 weeks. Early this week, more than 290 people were in isolation wards, according to the health ministry, which is supported by the World Health Organization and the U.S. Centers for Disease Control and Prevention (CDC). Two suspected cases in neighboring Kenya have both tested negative.

Uganda has seen Ebola outbreaks in 2000 and 2007 as well, with 425 and 149 cases respectively; in 2011 a 12-year-old girl died from the disease.

Raleigh 4

Sea Level Rise Bill Becomes Law

A controversial bill prescribing how North Carolina can forecast future sea level rise for planning became law on 2 August. Governor Beverly Perdue had until that date to act on the bill, known as House Bill 819, but she allowed it to become law by choosing to neither sign nor veto it.

Under the law, agencies are barred from considering accelerated sea level rise—such as might occur due to the melting of polar ice caps—in decision-making related to coastal development until 1 July 2016. Until then, the legislation requires state coastal planning agencies to base predictions of future sea level rise on a linear rate of increase as determined by "historical data."

"House Bill 819 will become law because it allows local governments to use their own scientific studies to define rates of sea level change," the governor wrote in a statement on 2 August. "I urge the General Assembly to revisit this issue and develop an approach that gives state agencies the flexibility to take appropriate action in response to sea level change within the next four years."

<http://scim.ag/NCSLbill>

NEWSMAKERS

Fermilab Director to Step Down

Pier Oddone, who has headed Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois, since July 2005, announced last week that he will retire on 1 July 2013. The announcement deepens the sense of transition for the U.S. particle physics community, which has faced a steady decline in funding and support



Oddone

for advanced accelerators in recent years. In September 2011, lab officials pulled the plug on the Tevatron, the last remaining U.S. atom smasher. In March, the U.S. Department of Energy, which funds Fermilab, announced that it could not afford the \$1.5 billion price tag for the Long-Baseline Neutrino Experiment (LBNE), and asked Fermilab physicists to come up with a plan to build LBNE in stages.

Still, Congress hasn't been as tight with its wallet as critics often make out, Oddone says; the bigger concern, he adds, is that recent administrations continue to prioritize applied research on topics such as energy and chemistry instead of basic physics.

A year from now, Oddone plans to focus on writing, advocating for science in a more direct way than he can do now, and working at a vineyard he owns in Sonoma, California, which grows grapes for Zinfandel and Cabernet wines. <http://scim.ag/Oddone>

Controversial Neolithic Archaeologist Dies

James Mellaart, a British archaeologist as celebrated as he was controversial for his research on early farming sites in the Near East, died on 29 July at age 86. Mellaart was best known for his 1960s excavations of Neolithic Çatalhöyük in south-central Turkey, a 9500-year-old farming village that may have been home to as many as 8000 people (*Science*, 20 November 1998, p. 1442). Mellaart claimed that Çatalhöyük was a major center of Mother Goddess worship, although many archaeologists now question that idea.

Mellaart was known for being flamboyant and sometimes exaggerating what he found. In the early 1960s, Turkish authorities began suspecting him of hiding a Bronze Age trea-

sure; that "Dorak Affair" led to Mellaart's being banned from Turkey in 1965, and he never excavated another site there or anywhere. Mellaart finished his career as a popular instructor at University College London's Institute of Archaeology.

"Jimmy Mellaart was a consummate field archaeologist and a gracious and ebullient man," says Ian Hodder, an archaeologist at Stanford University in Palo Alto, California, who has led new excavations at Çatalhöyük since the 1990s. "The discovery and excavation of Çatalhöyük were his greatest achievements."

Paris Researcher Rejects Legion d'Honneur Prize

French public health researcher Annie Thébaud-Mony declined to receive the Legion d'Honneur, the highest decoration in France, in her fight to protect workers from professional and environmental health hazards. Over the last 30 years Thébaud-Mony, now a semiretired research director of the

THEY SAID IT

"It's just a beat-up of a story and untrue."

—Australian billionaire mining magnate Clive Palmer, to *The Gold Coast Bulletin* on 1 August, knocking down rumors that he was working with the team that cloned Dolly to produce a resort with dinosaurs akin to the fictional *Jurassic Park*.

French National Institute of Health and Medical Research, has been investigating how working conditions, including exposure to industrial risk factors such as asbes-

tos and radioactivity, affect workers' health. Thébaud-Mony has also taken part in networks of researchers, doctors, lawyers, and affected workers militating against industrial hazards: She is >>



Thébaud-Mony



Touchdown! Curiosity Has Landed

Curiosity rover's 7 minutes of terror had the happiest of endings: The 500,000 lines of computer code went off without a glitch. The 76 onboard explosive devices popped off in sequence to the microsecond, throwing valves and cutting loose tether lines. From 340 kilometers away, the HiRISE camera on the Mars Reconnaissance Orbiter observed the Curiosity rover inside its entry vehicle dangling from its parachute; the descent vehicle with the rover tucked inside would soon drop out.

And at 1:37 a.m. EDT, word came down: "Touchdown confirmed. We're safe on Mars."

Not smashing itself to smithereens was only one of Curiosity's achievements in the NASA rover's first day on Mars. Within minutes of touchdown, Curiosity sent back its first images: a flat, windswept plain in the middle ground rimmed near the top center and right by the wall of Gale crater about 20 kilometers away. And meanwhile, the science has already begun: The uniform size of the small gravel at the surface suggests material carried from the crater rim by water rather than debris blown out of nearby smaller impact craters.

<<<NEWSMAKERS

a founding member of Ban Asbestos France and currently presides over the Henri Pézerat Association.

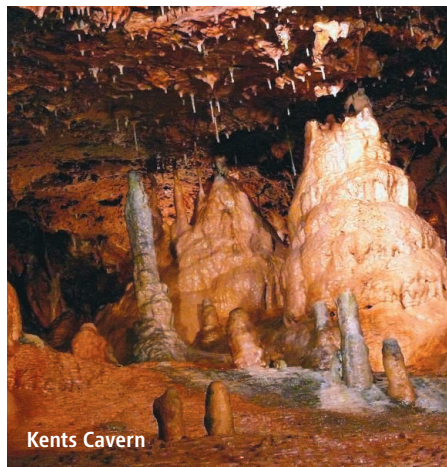
In a letter to housing minister Cécile Duflot made public on Saturday, Thébaud-Mony lamented that conditions for workers have deteriorated over the last 3 decades due to a climate of “terrible indifference,” and stated that researchers in the field have been lacking institutional support to conduct their studies. Rather than being offered a national decoration in recognition of her work, Thébaud-Mony wrote, she would prefer that the minister “challenge the impunity that until now has protected those who carry out industrial crimes.”

FINDINGS

The Mysterious Affair at Kents Cavern

Last year, researchers reported that an upper jawbone found in 1927 in the United Kingdom’s Kents Cavern is the oldest modern human fossil in Europe. But a new study argues that the date of the bone may never be certain.

After dating animal bones found above and below the fossil, Thomas Higham of the



University of Oxford and colleagues reported that the jawbone is between 41,500 and 44,000 years old, and belonged to a modern human rather than a Neandertal.

But in a new paper in press, Paul Pettitt of the University of Sheffield and Mark White of Durham University blast Higham’s results, saying it’s unclear exactly where the jawbone was originally found.

Apart from the Kents Cavern fossil and some disputed Italian teeth, the oldest human fossils in Europe, found in Romania, are about 40,000 years old. If modern

BY THE NUMBERS

3.5 Ratio of global withdrawal of ground water relative to ground water input, according to a study in *Nature* this week. That “groundwater footprint” is driven by a handful of overexploited aquifers.

\$20 million Amount drug company Novartis will invest in a research center at the University of Pennsylvania to develop a cancer vaccine.

humans made it to Northwest Europe by 41,500 years ago or earlier, they would’ve entered Europe much sooner than thought and moved rapidly. The overlap between modern humans and Neandertals, who were already in Europe, would also increase.

Higham and his colleagues reject the critique that they may have misunderstood the cave’s stratigraphy. Taking movement into account, they note, they still came out with dates of at least 41,000 years.

<http://scim.ag/Kentsfossil>

Random Sample

An Ancient-American Starbucks?

Europeans exploring the American southeast in the 1600s wrote of a purification ritual practiced by the native people, involving dancing, vomiting, and large amounts of what the travelers called black drink (inset). The highly caffeinated tea was brewed from the shrub *Ilex vomitoria*, a species of holly. Researchers have now found the first direct evidence of black drink—in ceramic beakers at the ancient city of Cahokia, outside of what’s now St. Louis, Missouri. The finding hints at a trade network that flourished centuries before Christopher Columbus landed in the New World.

In its time, between about 1050 C.E. and 1350 C.E., Cahokia was the largest and most sophisticated metropolis north of Mexico. Archaeologists excavating at Cahokia have found fragments of distinctively patterned, mug-shaped beakers containing residues presumed to be from chocolate.

Archaeologist Patricia Crown of the University of New Mexico, Albuquerque, and her colleagues wondered whether the beakers had contained black drink instead. Reporting online this week in the *Proceedings of the National Academy of Sciences*, Crown and colleagues found that the holly-based drink had a characteristic ratio of caffeine to theobromine, a chemical found in chocolate; the drink also contained ursolic acid, which is not found in chocolate. Comparing the chemical signatures, the team showed that the beakers had indeed contained black drink—some 500 years before the Europeans described the brew in their journals.

The leaves must have been brought to Cahokia from the coastal region



between eastern Texas and Florida, 400 kilometers away, the researchers note—suggesting the drink had huge cultural importance. The beakers were also found at possible ritual gathering sites. If the beakers and black drink go hand in hand, it might signify wide-ranging Cahokian religious influence between the 11th and 13th centuries. <http://scim.ag/Black-Drink>



U.S. SCIENCE FUNDING

Congress Ready to Extend Budget at Current Levels

U.S. government agencies waiting to know how much money they can spend in the fiscal year that begins in October got the word last week from Congress: We'll get back to you next spring.

Before heading out for a month of politicking, party leaders promised to draw up and pass a continuing resolution (CR) when they return. That budget measure would keep agencies at current spending levels until 1 April, halfway through the 2013 fiscal year. To some, that delay means immediate disappointment; to others, a second chance.

For the National Science Foundation (NSF), the delay jeopardizes its request for a 5% increase, which until this point has received strong support from legislators. The situation is more complicated for planetary scientists supported by NASA's

Mars exploration program and physicists in the domestic fusion program funded by the Department of Energy (DOE). The Obama Administration had proposed deep cuts to those programs, which congressional panels had recommended overturning; the CR puts off a decision until the spring. Likewise, the temporary agreement rescues long-running federal activities that the House of Representatives has targeted for elimination, including the American Community Survey (ACS) from the Census Bureau, NSF's political science portfolio, and economic research supported by the National Institutes of Health (NIH).

This is not the first time Congress has sidestepped its constitutional obligation to adopt an annual federal budget. In fact, it's been several years since Congress actually

passed individual spending bills drafted by the 12 panels that oversee the \$3.5-trillion-a-year federal budget. That process allows legislators to fine-tune an agency's request. But that fine-tuning is not possible under a CR, which amounts to simply waving a yellow flag at the entire budget.

Agencies have had lots of experience operating under such a cautionary flag, although this one comes earlier than in past years and extends for an unusually long time. NIH, for example, has typically funded only 90% of the approved budget of continuing awards made while the interim spending measure is in place to provide a financial cushion in case the final bill imposes actual reductions.

The stopgap spending measure is a byproduct of last summer's agreement to avert a federal default in that it pegged total spending for discretionary programs at \$1.043 trillion in 2012 and nearly the same amount—\$1.047 trillion—in 2013. But this spring, House Republicans, pressured by new members who had promised to slash federal spending, lowered the 2013 target by \$19 billion. Meanwhile, the Senate, controlled by Democrats, has been passing bills pegged to the higher level. While spending measures are always contentious, the different totals assured political gridlock.

With their public approval rates at rock bottom, however, neither house of Congress wanted to be blamed for a government shutdown caused by their failure to pass some type of 2013 spending bill. Republican leaders signed onto the deal because they expect voters to give them a mandate for deep spending cuts by sending presidential candidate Mitt Romney to the White House and handing them control of both houses of Congress. Democrats wanted to preserve the higher spending level for 2013, and they are betting that President Barack Obama will be reelected and that they will hold on to the Senate.

So what does all this mean for science? The first clues will come next month, when legislators decide whether to make any exceptions to the traditional CR language

COUNTDOWN TO CONFRONTATION?

30 September 2012	6 November 2012	2 January 2013	3 January 2013	21 January 2013	31 March 2013
End of 2012 fiscal year/start of continuing resolution	National election	Proposed "sequestration" cuts take effect	Opening of 113th Congress	Presidential inauguration	End of 6-month 2013 CR

that prohibits agencies from so-called new starts, that is, any deviations from the status quo. Although there's no guarantee they will do it, congressional leaders have pledged to write a "clean bill."

"I don't anticipate that any policy issues will be addressed, including ACS," says Timothy Maney of the U.S. Chamber of Commerce, one of many groups that have lobbied heavily for the Senate to reverse the House's elimination of ACS (*Science*, 15 June, p. 1367). "We'll continue to work the issue, but it's on the back burner for now." Michael Brintnall, executive director of the American Political Science Association in Washington, D.C., says the temporary agreement "gives us more time to focus on our long-term strategy. So that's welcome news."

U.S. CONFLICT REGULATIONS

Scientists Sue to Halt Financial Disclosure Rule

Outraged over what they see as an illegal invasion of privacy, researchers at the U.S. National Institutes of Health (NIH) and NASA have joined a lawsuit to block a provision in the Stock Act, a law enacted in April that will require federal agencies to post online financial reports for high-level government employees. The rule violates employees' rights, they say, and may drive some scientists to leave the government.

"We believe the Stock Act's extension to scientists is unconstitutional," says Joshua Zimmerberg, a lab chief at the National Institute of Child Health and Human Development (NICHD) in Bethesda, Maryland, and a plaintiff in the suit. In a statement appended to the lawsuit filed last week in a Maryland federal court, the biophysicist declares, "There is no question that the Stock Act will impact the ability to recruit capable scientists to public service." Zimmerberg states that he knows of individuals who have resigned or have declined to apply for an NIH job that entails the online disclosure rule.

The protest targets the Stop Trading on Congressional Knowledge (Stock) Act, intended to prevent insider trading by members of Congress. One provision of the law requires that a financial report known as

For Earl Marmar, director of the Alcator C-Mod fusion reactor at the Massachusetts Institute of Technology in Cambridge, passage of the 6-month CR will be the starting point for negotiations with DOE officials on the lab's fate. The department proposed a 16% cut in the domestic fusion program, with no money to operate the MIT reactor, and the House reversed that decision. "We'll try to find a way to continue operating the lab," he says. The facility has a staff of 120 plus collaborators across the country and around the world.

A CR is hard on a young agency like DOE's Advanced Research Projects Agency-Energy (ARPA-E), which opened its doors in 2009. The Obama Administration sought to boost its budget by 27%, to \$350 million, and

"new starts" are synonymous with its goal of funding potentially transformative ideas for novel clean-energy technologies.

So would a CR, for example, allow for a 2013 initiative on electrofuels? "We'll have to have a conversation with our appropriators," says a senior ARPA-E official. "You don't want to poke the bull." ARPA-E officials are in a delicate position: Splitting the difference between proposed House and Senate spending levels for the agency would give it a 2013 budget of \$255 million, not far from its current \$275 million.

But that decision, and many others, are now up to the next Congress, which convenes in January.

—JEFFREY MERVIS

With reporting by Jocelyn Kaiser and Adrian Cho.



"We believe the Stock Act's extension to scientists is unconstitutional."

—JOSHUA ZIMMERBERG,
NICHD

an OGE-278—in which members of Congress and their staff describe their assets and nonfederal income, as well as those of their spouses and dependent children—be posted on the Internet. Late in crafting the bill, lawmakers approved an amendment extending this reporting rule to some 28,000 executive branch employees who file an OGE-278. "It only seems fair" that they fall under the rule, argued the amendment's sponsor, Senator Richard Shelby (R-AL). The OGE-278 filers include scientists at NASA, the National Science Foundation, and other federal agencies, including about 600 senior staff members (not all scientists) at NIH in Bethesda, according to Holli Beckerman Jaffe, director of NIH's ethics office.

NIH researchers are already held to stringent limits on owning drug company stock, put in place 7 years ago. Their OGE-278 forms, which are reviewed by ethics officials, can be released to the public upon request. But the Stock Act mandates that starting on 31 August, these forms will also be publicly posted online. The forms must also be part of a searchable public database by October 2013.

In the motion filed last week, the Senior Executives Association and other plaintiffs

represented by the American Civil Liberties Union, including a group called the Assembly of Scientists representing 45 NIH and other federal researchers, argue that they and the public "will suffer immediate and irreparable harm from the online disclosure of Plaintiffs' private financial information." The employees say that making the forms easily available to anyone could make them vulnerable to identity theft, fraud, or even kidnapping.

Another researcher-plaintiff, Phil Skolnick, who left Eli Lilly in 2010 to head a drug-development division at the National Institute on Drug Abuse, says that "if I had known this [form] would be publicly posted, I would never have come here." NASA Chief Engineer Michael Ryschkewitsch filed a statement for the plaintiffs saying that some of his employees have asked to move down the federal pay scale to avoid falling under the Stock Act.

The suit seeks a preliminary injunction to block the requirement that financial reports be posted online. Last week, Congress approved a temporary reprieve, delaying the date for posting the OGE-278 forms by 30 days. Lawmakers said they need time to revise the law to protect national security; several former high-level federal officials have warned that it could harm the safety of defense, foreign service, and intelligence officials abroad. At presstime, the bill granting the delay was expected to be signed by President Barack Obama. Zimmerberg hopes the delay will also allow for "public discourse" about researchers' concerns.

—JOCELYN KAISER

OIL RESOURCES

Are World Oil's Prospects Not Declining All That Fast?

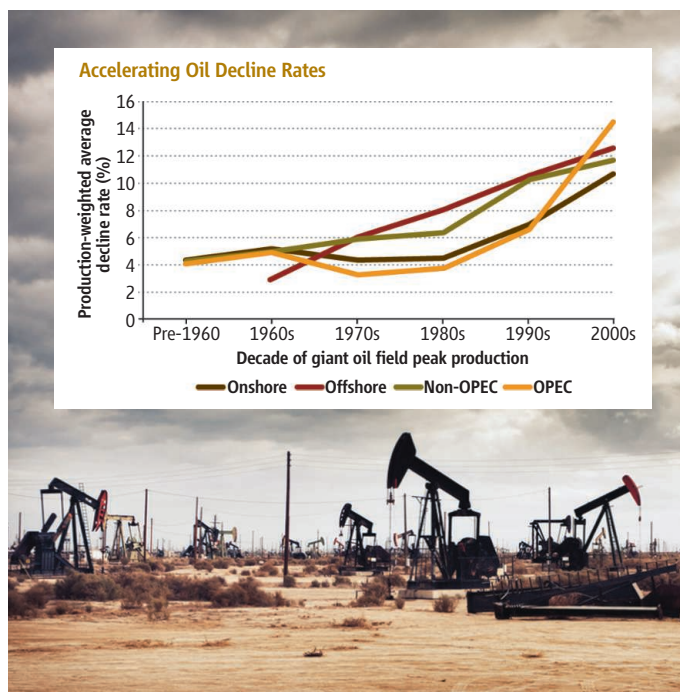
Lately the buzz in the oil patch has been all about growing abundance. New, more capable technology is coming on line: mining the oil sands of Alberta, wringing oil from beneath North Dakota by fracking, drilling down to the superdeep deposits beneath the Gulf of Mexico. Then, in June, a new report called *Oil: The Next Revolution* (<http://scim.ag/LMaugeri>) from a Harvard University researcher really fanned the flames of oil optimism. “Things have changed fundamentally and forever,” declared the editors of *Oil & Gas Journal*, the oil industry’s leading publication, on the release of the Harvard report.

But the report’s upbeat message depends on more than confidence in that fancy—and expensive—new technology. Overall, output from the world’s producing oil fields is always declining. To meet demand, drillers must continually find and tap whole new fields or pockets of old fields that had gone undetected. How much new production will need to be added depends on how fast the world’s fields are declining. The Harvard report estimates that output from currently producing fields will decline only half as fast as reported in recent decades. But energy analyst Steven Sorrell of the University of Sussex in the United Kingdom says “that’s wrong.”

The report’s newfound optimism about the decline rate derives from the singular analysis made by its sole author, Leonardo Maugeri. An economist, a former top manager at the Italian oil and gas giant Eni S.p.A., and currently a fellow at the Harvard Kennedy School’s Belfer Center for Science and International Affairs, Maugeri drew on his own proprietary database developed during his career in industry. He compiled data on 1100 fields in 23 countries that accounted for more than 80% of current oil production. The database for each field included past production (a relatively well-established number), planned future production, and the investments committed to achieving that production.

By doing field-by-field analysis, Maugeri came up with encouraging news about the decline rate. Major studies in 2008 by the

Paris-based International Energy Agency and by IHS Cambridge Energy Research Associates (IHS CERA), a consulting firm, estimated that production from existing fields had declined over recent decades at 4.1% per year and 4.5% per year, respectively. But “I still maintain it’s no more than 2%,” Maugeri says. “The historical evidence shows that decline rates announced in the past failed to explain even the current level of production.” Maugeri sees world crude oil production soaring from today’s 78 million barrels per day to 86 million barrels per day by 2020.



Although well regarded by industry, Maugeri’s report—especially its decline rate—has not been so warmly received beyond the oil patch. In a paper in the January 2012 issue of *Energy*, Sorrell and his colleagues reviewed the two 2008 decline rate estimates as well as an independent one published in 2009 in *Energy Policy* by Mikael Höök of Uppsala University in Sweden and colleagues. Sorrell and colleagues found that the three estimates were in reasonably good agreement and that the “decline rate of all currently producing fields is at least 4% per year,” twice Maugeri’s estimate.

A decline rate of 4% per year would mean that new production equal to that of Saudi Arabia—currently about 9 million bar-

rels per day—would have to be added every 3 years just to maintain production at current levels, Sorrell and colleagues noted. That’s a lot to ask, they say, even from the new technology. And it gets worse, they argued. The reviewed studies all predict that the decline rate will increase as giant fields discovered decades ago go into steep decline and drillers tap smaller fields and offshore fields, all of which decline faster (see figure). At best, keeping production constant “is likely to prove extremely challenging,” they wrote.

How did data from much the same fields yield both an upbeat outlook and a fair amount of doom and gloom? “In some oil fields, the gap between most analyses and mine can be explained by the use of new technologies,” Maugeri says. “Many fields are now going through a process of technological renovation.” Better technologies for identifying overlooked pockets of oil in and around known fields and for extracting more oil from the rock are shrinking the decline rate, he says. Combined with technologies opening up previously inaccessible oil deposits like the Canadian oil sands and North Dakota tight oil, a lower decline rate makes for abundant future supplies, he says.

Sorrell and others have been critical of Maugeri’s low decline rate. “Maugeri has made some very optimistic assumptions about global average decline rates, failed to provide adequate justification for them and misrepresented the estimates made by others,” Sorrell and Christophe McGlade of University College London wrote in a 6 July online posting (<http://scim.ag/Oilcomments>). IHS CERA’s Peter Jackson in London adds, “Ours was a very straightforward analysis looking at the production histories of 2000 to 3000 fields. There’s not much you can do to that to change the trends.” A subsequent expansion of the IHS CERA study last year found the same relatively high decline rate, he says.

The ultimate arbiter will be the drill. If decline rates are indeed low, new technology—from the Canadian sands to the Brazilian offshore and beyond—should boost the crude oil output of countries outside the Organization of the Petroleum Exporting Countries (OPEC). But so far, that hasn’t happened. Despite encouragement from high prices, non-OPEC crude production has been flat since 2003.

—RICHARD A. KERR



Intrepid reformer. Yang Fujia aims to start a liberal arts college featuring small classes and abundant student activities.

HIGHER EDUCATION

With Eye to Innovation, China Revamps Its Universities

SHANGHAI, CHINA—For nearly 2 decades, Yang Fujia has been dreaming about building a world-class university in China. His vision may at last be within reach. Yang is establishing a private liberal arts college in Tianjin, with enrollment to start, he hopes, in the fall of 2013. In crafting his new institution, the 76-year-old nuclear physicist-turned-educator, who was president of Fudan University here from 1993 to 1999, is taking cues from top Western universities. Students at the yet-to-be-named institution will live in University of Oxford-style residential colleges, attend small classes, and participate in self-organized activities. Yang also hopes to correct what he calls “the biggest problem in Chinese higher education”: Most Chinese universities, he says, focus on teaching students skills rather than nurturing their personal development.

Once, such a venture would have been unthinkable in a country with a tightly controlled education system. But Yang’s proposal comes at a propitious time: The Chinese government is embarking on reforms directed at turning out more creative graduates. Though the Tianjin project has yet to receive a green light from the education ministry, it has the blessing of Premier Wen Jiabao, a Tianjin native who in the spring announced that the government is encouraging the establishment of liberal arts colleges, according to Xinhua, the state news agency. Since then, Tianjin municipal government has offered 68 hectares of land for the college.

China’s leaders are “interested in experimenting with new models of under-

graduate education that can help students develop their creative potential,” says Jeffrey Lehman, vice chancellor of a campus that New York University is building here with East China Normal University, called NYU Shanghai. As part of a comprehensive 10-year overhaul, the education ministry is rethinking the admissions process, encouraging universities to carve out unique identities, and raising the level of basic research. A series of ministry directives earlier this year called for an increased reliance on the private sector and foreign partners to turn out more well-rounded graduates and boost China’s capacity for innovation. But the reforms leave intact the Communist Party’s involvement in higher education and do not relax restrictions on “thought education”: required courses designed to mold students’ political philosophy.

In the late 1990s, China began revamping and expanding its higher education system. Based on the notion that world-class universities are comprehensive, then-State Council Vice Premier Li Lanqing ordered the merger of specialty colleges and vocational schools into sprawling regional institutions with tens of thousands of students. Enrollment mushroomed from some 4 million undergraduates in 1999 to more than 22 million in 2010. But outside the top echelon, standards declined. “Quality can become abysmal with a break-neck pace of expansion,” says Gerard A. Postiglione, an education scholar at the University of Hong Kong. In China, he says, fall-out from expansion prompted a drive toward “boosting indicators of quality.”

In the meantime, the pool of high school graduates has shrunk. The number of students taking the college entrance exam peaked at 10.5 million in 2008 and has since declined to 9 million this year. Compounding the problem is that more and more Chinese are going abroad for undergraduate studies. In the United States, the number of Chinese undergraduates enrolled in the 2010–2011 academic year was 56,976—a roughly 43% increase over the previous year, according to the Institute of International Education in New York City.

The latest reform program, which charts goals to 2020, aims to make Chinese universities more competitive. This time around, the focus is on experimenting with new colleges and universities rather than consolidating existing ones—and encouraging older universities to distinguish themselves from the pack. Institutional branding is a global notion, Postiglione says. Many midtier universities in North America have successfully carved out a distinctive character, he says, by emphasizing institutional history or areas of academic strength.

China hopes its universities can do the same. One venture here wants to distinguish itself from the start: The new Shanghai University of Science and Technology, which received education ministry approval in April to prepare for student enrollment in 2013, aims to be China’s California Institute of Technology, says Jiang Biao, head of the preparatory group.

Along with piloting new homegrown institutions, the education ministry has endorsed projects spearheaded by foreign universities. Among those is NYU Shanghai, which will enroll students starting in fall 2013 and, like Yang’s project, is conceived as a liberal arts college. Another is the Joint Institute, an engineering school established here by the University of Michigan, Ann Arbor, and Shanghai Jiao Tong University. The curriculum is patterned after Michigan’s: In addition to core technical courses, students will be trained in ethics, creativity, and problem-solving skills. Administrators hope the Joint Institute will one day “be compared with the top 20 engineering schools in the U.S.,” says Robert Parker, associate dean for academic affairs. The institute has a powerful fan. State Councilor Liu Yandong, China’s highest-ranking science and education official, had a case study of the project disseminated earlier this year to other univer-

sities to encourage them to experiment with reforms, according to Parker and others.

But the ministry faces a challenge as it targets one pillar of the system: the high-stakes 2-day university entrance exam, or *gaokao*, required by all accredited undergraduate universities and colleges in mainland China. Though critics have long blamed the *gaokao* for promoting rote learning and stifling creativity, few advocate scrapping the test altogether. Founding President Zhu Qingshi of South University of Science and Technology of China, or SUSTC, sparked criticism when he bypassed the *gaokao* to admit the first batch of students (*Science*, 8 April 2011, p. 161). Many educators say *gaokao* reform should not ditch the test but allow universities flexibility in using test results for admissions. Zhu backtracked after SUSTC received ministry accreditation, opting to consider *gaokao* scores alongside high school grades and results of the university's own test. Last week, Liu urged a newly formed panel of 26 experts to come up with a plan to reform the content and format of the *gaokao* and give universities autonomy in admissions.

Overshadowing the experiments is the question of academic freedom. The reform plan reaffirms the requirement that college students take standardized courses with titles such as "Introduction to Mao Zedong Thought" and "Deng Xiaoping Theory." Foreign degree programs may be given more leeway; Lehman says NYU Shanghai "has been assured complete academic freedom." While classroom discussions at some universities can be quite open, students still face restrictions on starting independent publications and organizations. In order to remain operational, Postiglione cautions, foreign universities with campuses in China will have "to adapt to China's version of academic freedom."

Advocating for his liberal arts college, Yang did just that. In materials he distributed to government officials, he translated parts of Yale University's Report of 1828, which argues for a broad and standardized curriculum. But he omitted a sentence from the concluding paragraph describing the need for liberal arts in a nation where "a free government gives full liberty to the human intellect to expand and operate." Whether China's ambitious education reforms achieve their underlying goal of producing innovators may ultimately be determined not by residential colleges, small classes, or research funding, but by something that cuts to the soul of the nation.

—HAO XIN

With reporting by Mara Hvistendahl.

PALEOANTHROPOLOGY

A New Face Reveals Multiple Lineages Alive at the Dawn of Our Genus *Homo*

Ever since paleoanthropologist Meave Leakey got her first look at the skull of a strange new kind of human ancestor in 1972 at Koobi Fora, Kenya, she and others have searched in vain for more members of this enigmatic species. The 2-million-year-old skull had a big brain that made it a member of our own genus *Homo*. But its long, flat face and other features distinguished it from the other two members of early *Homo* known at the time, so many researchers thought of it as a new species, *Homo rudolfensis*. But some questioned whether it was a new species or just an unusual member of *Homo habilis*, which lived 2.3 million to 1.4 million years ago. "It was always an anomaly," says Leakey, of the Turkana Basin Institute in Kenya and Stony Brook University in New York. "We always knew we had to find more of it."

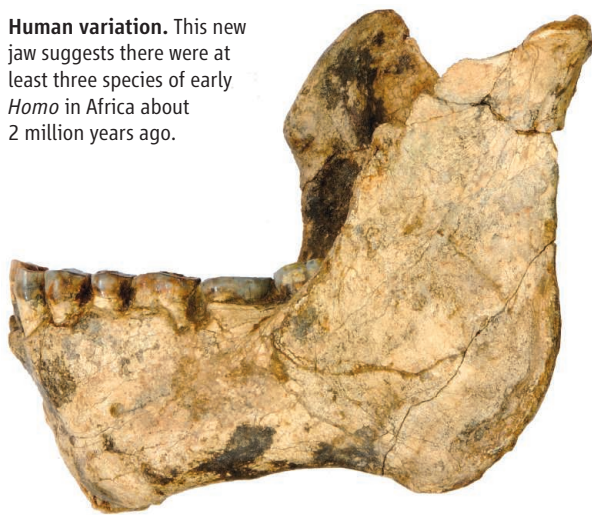
Now, after 40 years of searching, Leakey and her international team of researchers have found fossils of a face and two jawbones that they say belong to the same species as the mysterious skull of *H. rudolfensis*. Like the first find, the new fossils were unearthed at Koobi Fora on the east side of Lake Turkana. Together, they show that the original skull wasn't a "weird individual," and that a third type of early *Homo* did indeed live 1.78 million to 2.03 million years ago at Koobi Fora, says Fred Spoor of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, co-author of a paper on the specimens published this week in *Nature*. That means multiple lineages of early *Homo* lived at Koobi Fora at the same time as our direct ancestor *Homo erectus*, which lived about 1.8 million to 500,000 years ago in Africa and Asia. "This material shows there is really good evidence that there has to be *H. erectus* plus two or three other taxa," says paleoanthropologist Bernard Wood of George Washington University in Washington, D.C., who is not a co-author.

When the first small bit of jaw was found in 2007, Leakey didn't think it was particularly special. But then Elgite Lokorimudang of the Turkana Basin Institute found the well-preserved face and teeth of a juvenile protruding out of rock in 2008. This "was really exciting," Leakey says. It looked like a small

"pocket version" of the *H. rudolfensis* skull, known as KNM-ER 1470—with an unusually flat face, as opposed to the more jutting upper jaw found in *H. habilis*, Spoor says. Its small size also ruled out the older view that *H. rudolfensis* skulls were invariably larger than those of *H. habilis*.

With the discovery of a remarkably complete lower jaw in 2009, the team got its first good look at the jaw of this elusive species (1470 did not have a lower jaw). This and the new face revealed that *H. rudolfensis* had an unusual U-shaped palate, with canines fac-

Human variation. This new jaw suggests there were at least three species of early *Homo* in Africa about 2 million years ago.



ing the front of the jaw rather than aligned on the sides in a V-shaped palate, as in *H. habilis*. The new jaws also had smaller molars than expected; other flat-faced taxa, such as *Paranthropus boisei*, had huge molars. "Now we know: Having a flat face doesn't equal having big molars," Spoor says.

The new fossils were all found on the Karari Ridge of Koobi Fora, within 10 kilometers of the fossil beds where the 1470 skull was found—and within the same region where fossils of *H. habilis* and *H. erectus* have been discovered. Paleoanthropologist Timothy White of the University of California, Berkeley, warns that the new fossils could be *H. habilis* because "we still don't understand *H. habilis*." But if three species coexisted at roughly the same time and place, says paleoanthropologist William Kimbel of Arizona State University, Tempe, who is not a co-author, "we need to think about hypotheses to explain how they might have divided up their world adaptively." —ANN GIBBONS



Drained of life. A fungal disease has almost wiped out the mountain yellow-legged frog population in Kings Canyon National Park in California.

Attack of the Clones

Fungi have long been seen as the least interesting pathogens, but two catastrophes in the animal world have changed that view

WHEN NATURE RECENTLY ACCEPTED A review co-authored by Sarah Gurr, the plant pathologist from the University of Oxford in the United Kingdom sent the journal a self-produced image to consider for its cover. It shows a fungus looking like one of those colossal, menacing tripods from H. G. Wells's *War of the Worlds*, stalking through a field, with bats, frogs, and toads fleeing before it in a crazed panic. "Fungal Wars of the World," Gurr called it.

The picture didn't make it, but many scientists agree with its message: Fungi have now become a greater global threat to crops, forests, and wild animals than ever before. They have killed countless amphibians, pushing some species to extinction, and they're threatening the food supply for billions of people. More than 125 million tons of the top five food crops—rice, wheat, maize, potatoes, and soybeans—are destroyed by fungi every year.

Like other infectious agents, fungi benefit from a combination of trends, such as increased global travel and trade, new agricultural practices, and perhaps global warm-

ing. But they have several unique features, researchers say—including the way they can switch from asexual to sexual reproduction—that enable them to exploit these opportunities particularly effectively.

The *Nature* paper, published in April, was in part a cry for attention; its authors say the world isn't fully aware of the dangers and should invest more in countermeasures. For decades, fungal diseases have been overshadowed by bacteria and viruses. "There are probably 50 or 100 bacterial experts for every fungal expert," says Bruce McDonald, a plant pathologist at the Swiss Federal Institute of Technology in Zurich. "There has always been a sense that fungi are not that important," adds microbiologist Arturo Casadevall of Albert Einstein College of Medicine in New York City.

That has begun to change only very recently, thanks in part to some highly publicized animal die-offs. "A few years ago, people just scoffed when you thought a fungus had killed an animal such as a bat," says Gudrun Wibbelt, a veterinary pathologist at

the Leibniz Institute for Zoo and Wildlife Research in Berlin. "That is clearly changing." In December 2010, the U.S. Institute of Medicine hosted its first-ever workshop focused exclusively on fungal diseases, which concluded that "threats posed by emerging fungal pathogens are often unappreciated and poorly understood."

Interest in the *Nature* review has been "huge," Gurr says. Scientists around the world have sent in articles describing other fungal diseases that could have bolstered the paper, says co-author Matthew Fisher, a molecular epidemiologist at Imperial College London. Among the wide variety of species under attack are crabs, corals, corn, and the Cavendish banana—and new fungal diseases are discovered every year. In June, Elsevier presented a new journal called *Medical Mycology Case Reports*, completely devoted to "unusual medical or veterinary fungal infections."

Detective work

One of the issues that has held back research on fungi is that it's simply very hard. Fungi are more complex, have bigger genomes, and are more difficult to characterize than viruses or bacteria. In fact, most researchers find it hard to give a good definition of the whole group. ("That's tough. Let me have a quick look what Wikipedia says," one of them says.)

Until the 1960s, fungi were considered

to be closely related to plants, but scientists now know that they have a more recent common ancestor with animals—which means the mushroom in your salad is more closely related to you than to the lettuce. Like plants, animals, and bacteria, fungi comprise their own kingdom, which includes yeasts, molds, and mushrooms.

But the diversity is enormous—and most of it is uncharted territory. “We know the names of about 70,000 different fungi, but there are probably between 1.5 [million] and 5 million species out there,” Gurr says. Modern sequencing technology has multiplied the number of members in many fungal families. The number of known *Phytophthora* species, which include the cause of potato blight, has doubled since 2000, says David Rizzo, a plant pathologist at the University of California, Davis. Its size is what makes the fungal kingdom so scary, Casadevall says: “There is a lot of biological potential out there.”

The increased recognition of fungal threats was fueled by two major animal crises: the massive decline in amphibian species and an explosive disease outbreak among bats in North America.

Scientists had been observing declines in the numbers of frogs, toads, and salamanders since the 1970s. But it wasn't until 1997 that teams investigating simultaneous waves of population declines in Australia and Panama closely examined large numbers of animals and finally nailed the culprit: a fungus they named *Batrachochytrium dendrobatidis*, and which belonged to a clade not known to infect vertebrates. “It was an extraordinary piece of detective work,” says Fisher, who started working on *B. dendrobatidis* shortly after it was discovered. The fungus seems to have appeared at about the same time in Australia, Central and North America, and possibly Europe, and is spreading rapidly, Fisher says.

B. dendrobatidis's spores live in streams and ponds and have a flagellum that enables them to travel short distances; animals are thought to be infected by direct contact with the spores or with other infected animals. The fungus has caused the greatest disease-driven loss of biodiversity on record; some areas in Central America have lost more than 40% of their amphibian species.

Then, in late winter 2007, researchers found thousands of dead little brown bats with a white growth on the muzzles and ears in five caves in upstate New York. The following winter, the disease showed up in 33 caves, and by early 2012 it had spread north to Canada, south into Alabama, and as far west as Missouri. Bat white-nose syndrome, caused by *Geomyces destructans*, has now killed more

than 5.7 million bats from six species in the United States. In May, biologists announced a seventh victim: gray bats. Some hibernation sites have lost their entire population; according to one study, the little brown bat—one of the most common bats in the United States—has a greater than 99% chance of going regionally extinct in the eastern United States within 16 years. Because bats pollinate some plants and eat pest insects, their value to



Hanging in the balance.
The little brown bat is one of seven U.S. species infected by *Geomyces destructans*.

U.S. agriculture has been estimated to be at least \$3.7 billion a year.

Meanwhile, more and more fungal diseases are being found. In just the past 5 years, scientists have discovered fungi affecting rattlesnakes, land crabs, avocado trees, cultured abalone, and the eggs of sea turtles, for example.

The question is whether fungi are really on the march; it could be that scientists are just paying more attention and have better tools to detect and characterize them. David Relman, an infectious disease specialist at Stanford University in Palo Alto, California, thinks it's both. Gurr says there are other, more objective indicators of an increasing threat. On ProMED-mail, an e-mail list reporting outbreaks of infectious diseases based on numerous sources, the proportion of fungal alerts increased from 1% to

7% between 1995 and 2010. A similar rise occurred in HealthMap, another disease monitoring program, started in 2007.

Food crises

Plants are the group worst hit by fungi. In the first half of the 20th century, a previously unknown fungus called *Cryphonectria parasitica*, imported to the United States with Asian chestnut trees, killed more than 80% of the 4 billion American chestnut trees. More recent examples of trees falling victims to fungi include pines in Canada, larches in the United Kingdom, and oaks in California.

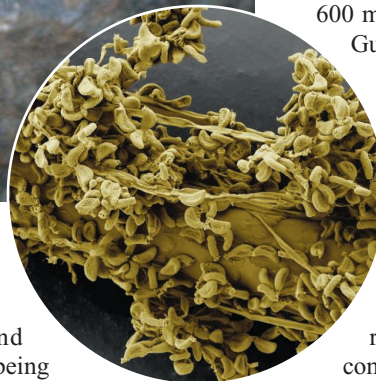
Fungal threats to the food supply—although as old as humanity—appear to be on the rise as well. Already, more crops are lost to fungal diseases than to viruses, bacteria, and nematodes together, Gurr says. Potato blight, caused by *Phytophthora infestans* (technically an oomycete, not a fungus, but usually classed with fungi as a fungal-like pathogen) caused the Great Irish Famine in the middle of the 19th century. Rice blast, wheat stem rust, soybean rust, and corn smut also threaten some of the most important crops; together with potato blight, they destroy enough food to feed 600 million people, according to Gurr's calculations.

Wheat stem rust caused some devastating food crises in history but seemed conquered after a new wheat variety including the stem rust resistance gene *Sr31* was planted from the 1960s onward.

In 1998, however, a stem rust clone that could overcome this resistance was found in Uganda. The strain, *Ug99*, has now spread across Africa to the Middle East (*Science*, 30 March 2007, p. 1786). “Pathologists really need to keep a good watch on the pathogen population,” says Ravi Singh, a plant pathologist at the International Maize and Wheat Improvement Center in El Batán, Mexico.

Flexible sex lives

Scientists blame increased trade, travel, and tourism for the new spread of fungal diseases—and fungi are especially well-placed to exploit these trends. Unlike most viruses and bacteria, they can persist for years outside the host, either as spores or living on dead matter, and they are everywhere. Exotic



Fungal victims. Chestnut blight (left) has killed billions of trees in the United States. Corn smut, potato blight, and wheat stem rust (right, top to bottom) threaten the world's food supply.

plant materials are routinely sent around the world. "People have not been aware that every time you send roses, you are sending fungi," Casadevall says.

Another advantage fungi enjoy is their very broad host range. "You cannot give HIV to your dog, but you can give *Cryptococcus neoformans* to a human, a mouse, an amoeba, a worm, or a plant," Casadevall says. *B. dendrobatidis* infects more than 500 amphibian species, in some cases without causing disease. It was probably spread across the globe in the first half of the 20th century with the African clawed frog, widely traded because it was a useful model organism in biology labs. Spelunkers may have carried the white-nose syndrome fungus to the United States from Europe, where it lives on bats but doesn't kill them (*Science*, 8 January 2010, p. 132).

In crops, other factors are at play as well. Since the green revolution, agriculture has been dominated by genetically identical staple crops grown on huge swaths of land, often protected against the most devastating fungal diseases by only one dominant resistance gene. Many fungi have very big genomes; *G. destructans* has more than 30 million base pairs, 10 times as many as the bacterium *Staphylococcus aureus*. Sequencing has shown that the number of genes is often quite small, Gurr says, but there is a vast amount of so-called retroDNA, genetic elements that can jump, which makes the genome very flexible and enables fungi to overcome resistance faster.

Modern agriculture has also removed the cost to a fungus of being highly pathogenic. In nature, a fungal disease that rapidly kills all members of its host species would soon face a dead end. By growing plants close together and using the same variety over and over, the fungus that reproduces the fastest leaves the most progeny. "We have given these fungi the most extraordinary opportunity to evolve fast," Gurr says.

Bananas are a prime example. In the 1950s, Panama disease, caused by the fungus *Fusarium oxysporum*, wiped out Gros Michel, the main export banana variety grown in the Americas. They were replaced by a Panama-resistant Asian variety called Cavendish. But around 1990, a new strain of the fungus called Tropical Race Four emerged in Asia that also infected the Cavendish. Because sexual reproduction has been bred out of bananas,



all the trees in a plantation—indeed, all Cavendish bananas in the world—are genetically identical, and one fungal clone could kill all of those cloned trees. If Tropical Race Four reaches Latin America, that could be the end of the Cavendish.

The way McDonald sees it, the key to fungi's success is that they reproduce asexually most of the time but switch to sexual reproduction when food runs out. "They have regular old-fashioned sex and through that put together new combinations of genes," he says. "When a good one comes together, they can explosively increase through asexual reproduction." The big epidemics are largely due to such single clones, McDonald says. Indeed, research published this year suggests that white-nose syndrome is caused by one clone of *G. destructans*, and while three clones of *B. dendrobatidis* have been described, only one of them seems to be responsible for the amphibians' plight.

So far, humanity's options to fight back against fungal threats to wildlife and crops are limited. Fisher and his co-authors call for tighter control of international trade in biological material to stem the spread of dangerous clones—although they acknowledge that this is hard in a world that is becoming ever more tightly knit together. "Every environment is now connected to every other environment on the other side of the world," Fisher says.

For crop diseases, there are other options. To help delay the emergence of resistance, farmers need to stop using fungicides that target only one molecule in the fungus, Gurr says. Similarly, breeders should develop new crop varieties that combine various resistance genes instead of relying on a single one. That is what Singh has been doing for wheat to protect it against Ug99. "We found that resistance

genes with a small effect are quite common. The issue is to select enough of them in a single variety, so you don't get much disease at the end of the crop season," he says.

There are also natural and synthetic molecules known as activators that boost the innate immune system of plants; essentially they mimic a pathogen attack on the plant. "But farmers do not like them because they consume a lot of energy and the yield goes down," Gurr says.

Meanwhile, scientists are focusing on basic research that might lead to entirely new ways to tackle fungi. For instance, they're studying the interaction between the pathogens and their hosts. Like bacteria, fungi seem able to shuttle effector molecules into host cells, weakening their immune responses. "That is an area that might yield important clues," McDonald says.

Raising the temperature

So what about humans? Are we under threat as well? In general, humans and other mammals are very well protected because most fungi cannot grow at the higher body temperature of warm-blooded animals. Bats succumb to *G. destructans* only when their body temperature drops to between 2°C and 6°C during hibernation.

For most of human history, fungi did not present a big threat to us or the animals we care about most, such as cows, pigs, cats, and dogs. That changed in the 20th century, when the HIV pandemic, transplantations, and steroid therapies caused millions of people to live with compromised immune systems. For them, fungi can be deadly. A 2009 paper in *AIDS* estimated that close to 1 million people annually develop cryptococcal meningitis, a fungal infection of the membranes covering the brain, and most die from it.

Casadevall believes humans may lose their edge as the world gets warmer. "It is very likely that the fungi will adapt" to higher temperatures, he argues. Organisms that are currently not pathogenic because they are not adapted to the human body temperature could make the jump.

If he's right, it would open up a whole new chapter in Gurr's war of the worlds.

—KAI KUPFERSCHMIDT

CREDITS (CLOCKWISE FROM TOP LEFT): DOUG KOONTZ/THE NEWS POST/AP PHOTO; ERIC SCHMELZ/USDA; NIGEL CATTLIN/VISUALS UNLIMITED/CORBIS (2)



Afghan elite. Graduates at American University of Afghanistan in Kabul celebrate, but their career plans are uncertain.

HIGHER EDUCATION

Can Afghan Universities Recover From War, Taliban, and Neglect?

The demand for technical talent and outside funding is helping colleges get back on their feet. But higher education is still not a priority

KABUL—Except for the armed guards in body armor at the entrance, on the roof, and patrolling the campus, this college graduation ceremony could be taking place anywhere. Students in black gowns and mortarboards are grinning for photographs with their families as the registrar hurries through the crowd, urging people to take their seats.

But once the keynote speaker takes the stage, the difference between this ceremony and countless others becomes clear. For starters, it celebrates one of the first graduating classes at Afghanistan's only elite private institution for higher learning, American University of Afghanistan (AUAF). And the speaker, chemist Akram Fazel, is asking the students *not* to follow in his footsteps.

To be sure, Fazel has enjoyed a first-class education and ample success. Indeed, he has lived the life many dream of. After gradu-

ating from high school in Kabul, Fazel left Afghanistan and eventually earned a Ph.D. in chemistry in the United States, at the State University of New York, Binghamton. He worked for Danone, the multinational food and cosmetics company, and rose to become director of research before starting his own biotech consulting firm based in Paris and Shanghai.

Speaking in Dari, one of the country's two main languages, Fazel says that the next generation of Afghan scholars should eschew globetrotting and put their expertise to work at home. "It is up to you to rebuild this country," he tells the 45 students who are receiving their bachelor's degrees on this scorching day in May.

Now chair of the board of trustees of this 6-year-old institution, Fazel knows as well as anyone that the tiny graduating class can't

satisfy the country's desperate need for technical talent. "After 3 decades of war, Afghanistan has lost one-and-a-half generations of experts in all fields," says Timor Saffary, the head of AUAF's department of science and mathematics. "The intellectual elites either left the country or have passed away, leaving a huge vacuum." Saffary earned Ph.D.s in both mathematics and physics and worked as a postdoctoral researcher in Germany before joining AUAF in 2009, and the 39-year-old native of Kabul hopes to be a part of the generation who fills that vacuum.

With the help of the United States and other countries, Afghan academics are beginning to pick up the pieces. But a deadline looms: International military forces are preparing to leave the country

by the end of 2014, and it's anybody's guess what will happen after they are gone. "Many of the highest qualified and talented academics are looking nervously at the 2014 withdrawal," says Michael Petterson, a geologist at the University of Leicester in the United Kingdom who leads field research and training workshops in the region. "And who can blame them?"

Afghan ivy

What returning Afghan academics have found is a once-proud system of higher education on the rebound after hitting rock bottom a decade ago. In 2002, Afghanistan had 12 barely functioning universities; now it has 30, and they enroll roughly 100,000 students. Secondary education has enjoyed an even more impressive recovery, with the number of high school graduates increasing sevenfold since 2002.

In fact, that surge has overwhelmed the country's system of higher education. Admission to public universities is based on a nationally administered exam, and students pay no tuition. The Ministry of Higher Education projects that, without a significant increase in capacity, universities will be able to offer spots to only one in 10 students who apply in 2014.

The anticipated leap in demand was one reason the government created AUAF as the country's only not-for-profit, private and independent university. The U.S. government is its main funder: The U.S. Agency for International Development (USAID) has spent \$200 million on higher education programs in Afghanistan since 2002, and half of its current tranche of \$90 million for program funds is designated for AUAF. USAID Administrator Rajiv Shah calls the school a "best-in-class institutional partner" and says the university is intended to show "the value of true, high-quality higher education in helping societies grow and develop."

Although AUAF operates under a charter from the government, an independent board of trustees sets its policies. At \$5500 a year, tuition is beyond the reach of all but the wealthiest Afghan families, and 70% of its students receive financial aid.

Sharif Fayez, a Persian literature scholar who helped launch AUAF as then-minister of higher education, says that a Western-style university is needed not just to attract the best students "but also the best faculty." A typical public university professor earns less than \$500 a month, while AUAF faculty salaries are competitive with those at Western universities. In return, all faculty members are expected to hold an advanced



Homeward. Muhammad Mujtaba plans to return to Afghanistan after U.S. graduate training.

ican University [in] Cairo [for] 20 years." That university had weaned itself from U.S. funding and was self-sustaining at that point, he adds.

AUAF has similarly lofty aspirations, and it's moved with deliberate speed in building its capacity. It currently offers students three undergraduate degrees, with the vast majority choosing business and finance. (Computer science/information technology and political science/public administration are the other two bachelor's-level programs.) There are seven faculty members in its science and mathematics department, which offers a variety of introductory and mid-level courses. Some 500 students are enrolled in its undergraduate degree programs, with a similar number pursuing English-language studies. Another 800 students are taking short courses in various fields offered by the university's professional development institute. Fazel says that chemistry and earth science degrees are next on his wish list.

A history of violence

But while AUAF may ultimately train the elites, the vast majority of Afghans seeking higher education will find it in the public university system. And that system is creaking.

Only a 10-minute drive away, Kabul University represents the yin to AUAF's yang on the circle of Afghan higher education.

Its leafy, walled-in campus serves as a quiet oasis in a city that struggles to provide even the most basic amenities—water, power, waste disposal—for its 5 million residents. Its 20,000 students make it by far the largest university in the country.

Founded in 1931, Kabul University is also the country's most prestigious, and its science programs are bulging at the seams. "This is introductory physics," says Mohammad Arif, a chemist and dean of the faculty of science, poking his head into a lecture hall. The sweltering, windowless hall, with hundreds of students crammed into every seat right up to the top wings, looks more like the setting for a rock concert than a physics class.

"We are at double capacity," Arif says. Some 1500 students are pursuing science and math degrees in the departments under his watch. The total does not include applied science majors in the university's schools of engineering, agriculture, and medicine.

The current situation is a far cry from the recent past, says the 62-year-old Arif, who has taught at Kabul for 2 decades. "In the days of the Taliban, it was normal to have only one or two students in our classes," says Arif, a cosmopolitan intellectual who was forced to wear a beard and turban during their reign. And that era was only the latest insult to the country's system of higher education.

Arif had just finished his Ph.D. in chemistry in Moscow in 1979 when the Soviet Union invaded his homeland. "That's when everything fell apart," he says. The departure of Soviet troops in 1989 led to a civil war that subsided when the Taliban took over. "We just never recovered."

The U.S. invasion in 2001 offered a ray of hope for Afghan academics. But so far job training has been the highest priority. "We actually put far more resources into vocational training in communities—essentially the Afghan version of community colleges," says Shah about USAID's portfolio.

The Afghan government spends about \$35 million a year on higher education, most of it for administration, faculty salaries, and routine maintenance. Other requests wind up in the back of the queue. Two years ago, for example, Arif applied to the ministry for funds to update the chemistry labs. "I still have not heard back," he says.

The other science disciplines are also languishing. "This is our physics lab," he says, unlocking a tiny room. Old and battered oscilloscopes, scales, and voltmeters are laid out at a dozen stations along the walls. "They were donated by Germany probably



Opportunity. Women engineers are taking advantage of an internship program for fourth-year college students.

degree. (The figure is 40% for all Afghan universities, up from 30% in 2008.)

"We've had a lot of success with this model over the course of USAID's history," Shah says. "It was actually the American University [in] Cairo that brought a lot of technology industries to Egypt. And a lot of the people who led the efforts in Tahrir Square were graduates of a social mobilization and technology class taught at the Amer-

30 years ago. We don't have enough lab equipment to teach the basics, and our textbooks are completely outdated."

It wasn't always so. During the Cold War, the United States and the Soviet Union competed with each other to invest in Afghan higher education. "The early 1960s was a golden age," says AUAF's Fayeze. There were academic exchanges and research collaborations with U.S. universities such as Purdue University, the University of Wyoming, and the University of Nebraska, Omaha. Columbia University went a step further, building an institute in Kabul to train future teachers. Fayeze was one of many Afghans in the program, which included a year in New York City.

Not to be outdone, the Soviet Union invested heavily in science and engineering. It helped to build up Kabul's polytechnic universities, and by the 1970s Afghan academics were shuttling constantly between Moscow and Kabul. One reminder of that partnership is the fact that the older generation of Afghan scientists and engineers, like Arif, are just as likely to speak Russian as English. But the Soviet invasion soured that relationship.

Problems as opportunities

Getting accurate demographic statistics in Afghanistan is notoriously difficult. But 3 decades of war have certainly exerted a heavy toll on the country. When U.S.-led forces arrived in 2001, Afghanistan ranked near the bottom of every global index for wealth, public health, and education. Girls were forbidden from attending school. While neighboring Pakistan managed to double its literacy rate during that period, to 50%, fewer than 20% of Afghans were literate.

A decade later, Afghanistan's problems remain, but the trends are more encouraging. Literacy has reached 28% and is climbing. Only a third of students are female, but the gap is narrowing. "In primary education alone, we've gone from about 2 million kids in school, with almost no girls, to 8 million kids in school, more than 3 million of whom are girls," says USAID's Shah. "I think those 3 million girls going to school every year is a huge accomplishment America should be proud of."

Stroll through Kabul and its problems are all too clear. Packs of feral dogs compete with begging children for street corners. Some of the larger roads now have gutters, but elsewhere rubbish accumulates like snow drifts. Fewer than 10% of the city's roads are paved, and stinging red dust min-



"Those 3 million girls going to school every year is a huge accomplishment America should be proud of."

**—USAID ADMINISTRATOR RAJIV SHAH,
AT SITE OF REBUILT GHAZI HIGH SCHOOL**

gles with the smog. Traffic accidents are too common to attract notice, with cars navigating almost entirely without rules or street signs. And this is the capital city, the epicenter and criterion for international funding.

Then again, it is exactly these problems that appeal to scientifically trained Afghans like Muhammad Mujtaba, an engineering graduate student at the University of Missouri, Columbia. "I would like to bring a real change to the environmental situation in Kabul," he says. He plans to return to Kabul next year to work as a civil engineer.

Agriculture is another area where, as Fazel says, "problems are also opportunities." For most Afghans, modern agricultural techniques, especially irrigation systems adapted to the country's complex, water-deprived environment, have barely arrived. "Agricultural engineering can make a huge difference here," Fazel says. There's also the challenge of finding a crop as profitable as poppy; Afghanistan is the largest producer of opium in the world.

And then there are the opportunities hidden underground. Foreign nations have been scrambling for the chance to share in the country's mineral wealth. The U.S. government announced in 2010 that geologic

surveys had found that Afghanistan has enough iron and copper to make it one of the world's top producers. A Chinese mining company was the first to secure digging rights, and a Chinese-owned copper mine in southern Afghanistan is expected to become the largest foreign commercial investment in the country when it opens in 2014.

But those activities won't generate many well-paying jobs for Afghans unless the universities can provide the appropriate training. "[We] lack the skills and preparedness for such work," says Nasir Shir, one of Afghanistan's leading geographers, who is based in Kabul and at the University of Maine, Portland. Afghan universities have embraced the challenge of generating the relevant expertise. Last year, for example, Kabul University began a master's degree program in physics, its first graduate program in the sciences.

Fazel says the Afghan government needs better scientific advice to take advantage of these opportunities. For the

past several years, he and a small group of Afghan scientists have been pushing for a national science council that would report directly to the Afghan president, along with a national research center that would focus on the country's top priorities: "agriculture, healthcare, mining, and [information technologies]." In spite of the group's influence and energy, however, the government has shown little interest in either idea. Short-term security has trumped longer-term priorities such as science, he says, and security is likely to remain a priority after the 2014 troop withdrawal.

Back on the AUAF campus, the graduation goes off without a hitch. As the families head home, the guards look relieved. Fazel is bothered by the razor wire topping the high walls. "I look at this and I feel ashamed. A university should be an open place," he says.

But the students take no notice. They gather on the lawn one last time for a celebration that would have been punishable by death only a decade ago: Pop music blares from a stereo and everyone dances. It's not rocket science, but it's a start.

—JOHN BOHANNON

With reporting by Jon Cohen in Washington, D.C.



PROFILE: JOÃO ZILHÃO

Neandertal Champion Defends the Reputation of Our Closest Cousins

Archaeologist João Zilhão and his critics trade charges over who truly invented artifacts at European sites—and whether Neandertals were “modern”

TORRES NOVAS, PORTUGAL—When João Zilhão was 14, he and other young spelunkers from Lisbon began exploring a labyrinth of caves in the cliffs overlooking this sprawling municipality in central Portugal. In those days, it took 5 hours by bus to get here, and there were few hotels in the area—certainly none that they could afford. So Zilhão and his comrades slept in the long, cool passageways of the Galeria da Cisterna.

Zilhão credits those experiences for his success as an archaeologist, for he followed the inner maze of the caves to some of the most important finds of his career. He found 7500-year-old artifacts from Portugal’s earliest known farming community in the Galeria. Then in 1989, he and a colleague climbed up a shaft in the Galeria’s roof through 40 meters of vertical passageways and discovered a hidden back entrance to another cave, the Gruta da Oliveira. There, Zilhão unearthed extensive Neandertal bones and tools, and the cave is now an important example of late Neandertal survival.

Today, Zilhão, 55, is well-known as the Neandertals’ fiercest advocate, taking on any and all suggestions that their mental abilities might have been inferior to those of modern humans. His slender, soft-spoken presence often contrasts with his biting criticisms of others’ viewpoints, especially in print. “Where he passes, scorched earth follows,” says one archaeologist who asked not to be identified.

In June, Zilhão, now at the University of Barcelona in Spain, scored a key point in this debate: He and colleagues reported in *Science* that paintings from at least one Span-

ish cave might predate the modern human occupation of Europe, making Neandertals the possible artists (15 June, p. 1409). For Zilhão, however, “possible” is not enough. The new dating, he insists, “implies a strong probability of Neandertal authorship.”

Such strong statements have become Zilhão’s trademark and make him a formidable foe in what some researchers call the “Neandertal wars,” the scientific debate over Neandertals’ intelligence and taxonomic status. Zilhão’s dogged insistence on the logic of his case makes him “a tough person to argue with, ... the kind of researcher that can be frustrating and even aggravating at times,” says fellow Neandertal defender, paleoanthropologist Erik Trinkaus of Washington University in St. Louis, Danforth.

Even Zilhão’s opponents agree that he is a force to contend with. He is “a top-notch archaeologist,” says archaeologist Harold Dibble of the University of Pennsylvania. Zilhão’s advocacy of close biological and cultural similarities between Neandertals and modern humans “has changed what we have to think about,” says archaeologist Iain Davidson of the University of New England in Armidale, Australia.

Into the caves

Zilhão was born in Lisbon, the son of an engineer father and a psychiatrist mother, and grew interested in history, archaeology, and politics early on. When he was young, Portugal was still in the grips of the fascist dictatorship of António de Oliveira Salazar, and like many of his peers, Zilhão spent his youth protesting against the regime, which

Where it all began. Zilhão found 30,000 years of Neandertal occupation in Portugal’s Gruta da Oliveira.

ended in 1974. He traces his rebellious nature and his sympathy for the underdog to those turbulent days.

At 14, he began visiting caves with the spelunking club at his high school. Because Portugal had no undergraduate archaeology degrees at the time, he studied economics at the University of Lisbon, but soon switched to history and spent summers volunteering on archaeological digs in Portugal and France, biding his time until graduate school at the university. For his 1200-page archaeology Ph.D. thesis, Zilhão synthesized the little-known Upper Paleolithic of Portugal, the heyday of prehistoric modern humans.

Portugal was still “peripheral” to mainstream archaeology in those days, Zilhão says, which gave him independence: “I didn’t have an old man telling me what I had to do and how I had to think.” Zilhão first worked as a professor at the University of Lisbon, where he successfully campaigned against a dam that would have flooded hundreds of rock art engravings in Portugal’s Côa Valley.

Then his team found Neandertal tools, hearths, and fossils dating from 65,000 to 35,000 years ago at Oliviera, making them among the latest surviving Neandertals—consistent with other evidence that Portugal and Spain served as a “refugium” for the last Neandertals.

In 1996, Zilhão entered the Neandertal wars in earnest. For decades, researchers had argued about the authorship of an Upper Paleolithic culture called the Châtelperronian, found largely in France and characterized by personal ornaments made of animal teeth and ivory rings. That year, a team led by anthropologist Jean-Jacques Hublin, now at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, published a pivotal paper in *Nature* concluding that the Châtelperronian had been made by Neandertals. But Hublin’s team concluded that the ornaments were the result of “acculturation” with modern humans, meaning that the Neandertals had either imitated modern human behavior or even gotten the ornaments through exchange or trade.

Zilhão was outraged. “This was an extraordinary conclusion,” he says. He saw it as a sign of bias against the Neandertals, an assumption that they were not capable of inventing the ornaments themselves. In 1998, Zilhão, archaeologist Francesco D’Errico of the University of Bordeaux in France, and others published a long paper in *Current Anthropology* challenging

the acculturation model and arguing that Neandertals had invented the Châtelperronian independently.

The paper rekindled a fierce and long-standing controversy over just how different Neandertals and modern humans really are, a debate whose flame has not yet dimmed. “This is an old debate and quite cyclical,” Dibble says. “At times the Neandertals have been considered to be a branch of stupid, stoop-shouldered hominins. ... Then the pendulum swings and they are considered to be exactly like us.”

Zilhão has continued to try to push the pendulum back, arguing vehemently against the acculturation model and insisting that Neandertals and modern humans are the same species and that they intermixed extensively. For example, in a 2010 paper in the *Proceedings of the National Academy of Sciences*, Zilhão and Trinkaus argued that the teeth of a child from the Lagar Velho rock shelter in Portugal show hybridization between moderns and Neandertals. Recently, the two have argued that the oldest undisputed modern human fossil in Europe, a roughly 40,000-year-old skull from Romania, bears some Neandertal features.

Zilhão sees the recent finding that many living people harbor between 1% and 4% of Neandertal DNA as vindication of this position. “That doesn’t mean that there was only 1% to 4% admixture back then,” he

Although some consider Zilhão biased, he accuses others of prejudice against Neandertals, recently commenting that some colleagues are on a “mission from God” to give modern humans credit for the Neandertals’ cultural accomplishments (*Science*, 1 June, p. 1086). And he insists that his viewpoint is gaining ground. “Twelve or 15 years ago, people like myself ... were in the minority, but now ... I don’t know a single individual actually involved in [Neandertal] research in Spain, Portugal, or France who thinks Neandertals were dumb or had inferior cognition to modern humans.”

Straw man?

But Zilhão’s critics are not swayed. They counter that Zilhão and his colleagues have created something of a straw man, because few serious researchers argue that the big-brained Neandertals were stupid. Neandertal



Symbolic species. Neandertals made this 40,000-year-old painted jewelry from the Antón rock shelter of Spain.

is less sophisticated than that of moderns. He and others see Neandertals and moderns as distinct species that were separated for hundreds of thousands of years. “Denying any form of biological differences [between them] is a sort of creationism,” Hublin says, an argument that “rejects the very phenomenon of evolution.”

And Hublin insists that Zilhão is the one who seems to be on a mission. “Those who are on a mission from God ... are those who try to deny any evidence not matching with their personal crusade,” Hublin says. “The latest debates on Neandertal abilities are one of the worst examples in which ideological issues have overshadowed scientific evidence.”

Dibble questions what he sees as an implicit tenet of Zilhão’s position, “the belief that it would be somehow insulting to the Neandertals to conclude that their behavior is not like ours.” Rather, he says, they used different kinds of adaptations to survive. “Neandertals were around for 250,000 years. That’s pretty successful, and better than we’ve done so far.”

Trinkaus says that both he and Zilhão have been “repeatedly accused” of being “politically correct” where Neandertals are concerned—treating them as a Stone Age minority group in need of affirmative action. But that’s a charge he rejects. “João is, and will remain, controversial,” Trinkaus says. “But over the past decade he has contributed more of substance, and more ideas, to [Paleolithic] archaeology than any other active participant. His legacy is already being felt, and he has many years ahead of him.”

—MICHAEL BALTER



Just like us? Zilhão and like-minded colleagues argue that Neandertals and modern humans belonged to the same species and were equally smart.

argues, “but it’s 1% to 4% of our genes today, 40,000 years after the fact.” He also argues that he already has produced the smoking gun on Neandertals’ symbolic abilities: jewelry, including ochre-painted shells, from sites in Spain dated as early as 45,000 to 50,000 years ago, earlier than the first accepted dates for modern humans in Europe (*Science*, 15 January 2010, p. 255).

acculturation during the Châtelperronian had “nothing to do with inferiority,” Hublin says. Cultural diffusion between groups “is one of the most common and well-documented phenomena in the ethnographic and historical record.” He and others see key differences in the skeletons and archaeology of Neandertals and modern humans—for example, Hublin says, the claimed Neandertal jewelry

LETTERS

edited by Jennifer Sills

Predicting the Next Influenza Virus

IN JULY, THE MEXICAN GOVERNMENT DECLARED A NATIONAL ANIMAL health emergency after an outbreak of highly pathogenic H7N3 influenza in poultry (1). Although predictors of such outbreaks have long been sought, prospective surveillance in wild aquatic birds (the main influenza A reservoir) has never provided early warning.

Our 35-year influenza surveillance data in North American wild aquatic birds (2) shows peaks of H7N3 activity that preceded or coincided with lethal outbreaks in poultry in Chile (2002) (3); the Fraser Valley of British Columbia, Canada (2004) (4); Saskatchewan, Canada (2007) (5); and, currently, the Mexican state of Jalisco (2012). In fact, H7N3 has been the culprit in all lethal influenza outbreaks in poultry in the Americas over the past decade. Furthermore, it poses a risk to humans. The virus was transmitted from poultry to humans in British Columbia (6), and humans were infected with highly pathogenic H7N7 and H7N1 viruses in the Netherlands (7) and Italy (8). The periodic transmission of H7N3 viruses in the Americas from wild aquatic birds to gallinaceous poultry suggests that

these viruses continuously circulate in nature, and their propensity to become highly pathogenic after transmission suggests that they have a gene constellation conducive to generating pathogenic variants.

Science has long sought a method of predicting the emergence of highly pathogenic influenza viruses that pose a threat to both agriculture and human health, and these long-term influenza surveillance findings indicate that it may be possible. Could such coincident data provide useful early warning of dangerous outbreaks? We believe so. Although thorough analysis of the genomics of the H7N3 virus is urgently needed to identify potentially highly pathogenic strains, long-term, comprehensive analysis of influenza surveillance data can be useful in warning of possible agricultural, and potentially human, outbreaks of serious disease.

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Steps Forward for Greece

A RECENT LETTER BY H. ZUR HAUSEN *ET AL.* ("Support for Greece," 25 May, p. 978) highlighted a petition signed by distinguished Nobel Laureates from all over the world and addressed to the heads of three European Institutions, asking them to provide support to Greece and in particular to its academic and research community, in order to mitigate the adverse effects of the financial crisis. On behalf of our members, the academic teachers and researchers of Greece, we are grateful to our esteemed colleagues for their support.

The austerity measures (salaries and budgetary cuts) imposed as a result of the recent financial crisis have jeopardized the sustainability of research infrastructure and intensified the emigration of both young talented researchers and senior prominent

staff. To reverse the decline in university functions and R&D activities in Greece, and to promote growth through technology and innovation, we recommend the following goals:

(i) Adopt a clear R&D strategy that is relevant to Greece's current financial and social problems, that draws on its history, culture, physical environment, and natural resources, and that has strong links to higher education, within a unified higher education and research system.

(ii) Make efficient use of European structural funds (1), establish a regular and predictable timetable for calls for research, and increase gradually

the proportion of GDP allocated to R&D and education, given that "investments in R&D and education increase the chances to smooth

NextGenVOICES

Big Ideas: Last Call

You have one more week to respond to the NextGen VOICES survey! Share your thoughts about this question:

What one big idea in your field do you wish that every non-scientist understood? Why?

To submit, go to http://scim.ag/NextGen_4.

Deadline for submissions is 17 August. A selection of the best responses will be published in the 5 October issue of *Science*. Submissions should be 250 words or less. Anonymous submissions will not be considered. Please submit only once.



Academy of Sciences, Athens, Greece

the adverse impact of the crisis" (2). Eliminate excessive bureaucratic procedures, such as complicated and time-consuming processes for the recruitment of research staff and multiple approvals for dispensing expenses.

(iii) Modernize the governance and function of Greek academic and research institutions. Speed up the implementation of the recent framework law for universities, which creates a University Council with oversight functions and limits the electoral bodies for the rector and other positions to faculty members (excluding students and administrative staff). Update the current legislative frame-

work for research, which lacks incentives for networking among tertiary education institutions, research centers, and innovation-driven enterprises.

(iv) Streamline the "architecture" of the higher education and research infrastructure by consolidating teaching, research, and administrative activities.

Meanwhile, the European Union should encourage innovation and facilitate Greek participation in relevant EU programs, such as the National Strategic Reference Framework (NSRF) 2007-2013 and the EU Framework Programme for Research and Innovation (Horizon 2020), by establishing more favorable conditions for funding.

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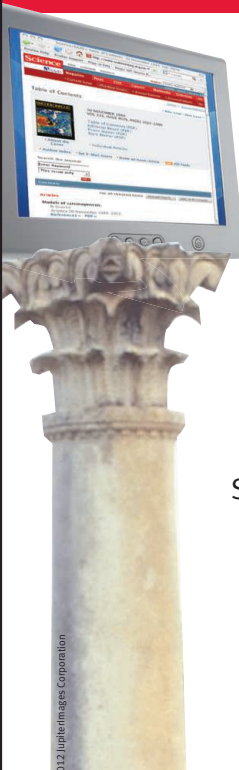
Mainstreaming Systems Science

IN HIS ESSAY "SYSTEMS SCIENCE FOR policy evaluation" (15 June, p. 1398), P. Kabat argued that we need to use systems science to inform better public policy. We agree completely. But why don't more sustainability scientists actively advocate and practice systems science?

First, scientific tradition has a preoccupation with reductionism. Reductionist science is of course vital to the scientific endeavor, but it is not the only way of doing science, and it is not necessarily more rigorous, valid, and reliable. Second, systems science involves higher transaction costs. It requires multidisciplinary research and, in ecosystem science, usually involves stakeholder participation.

Kabat correctly identifies the need to reshape academic merit systems so they

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reward integrated, multidisciplinary systems science. This change will help bring systems science into the mainstream of scientific endeavor. The scientific community needs to make a concerted effort to bring systems and reductionist science together to understand the interdependencies between the key global risks of our time, such as climate change, food security, biodiversity decline, economic disparity, and governance failures.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

CORRECTIONS AND CLARIFICATIONS

Editorial: "Iceberg alert for NIH" by H.R. Bourne and M.O. Lively (27 July, p. 390). The Editorial mistakenly indicated that 150 research faculty were laid off by the University of Miami's Miller School of Medicine. This sentence should have said that "researchers" were laid off. The text has been corrected in both the PDF and HTML versions online.

TECHNICAL COMMENT ABSTRACTS

Comment on "Impaired Respiratory and Body Temperature Control Upon Acute Serotonergic Neuron Inhibition"

Stefan Löffler, Jochen Körber, Udo Nubbemeyer, Karin Fehsel

Ray *et al.* (Reports, 29 July 2011, p. 637) assume that clozapine-*N*-oxide (CNO) represents a "biologically inert synthetic ligand" that selectively activates the M4 muscarinic receptor-based DREADD (designer receptor exclusively activated by a designer drug). In contrast, due to the redox cycling of CNO with clozapine and to their cell membrane permeability, CNO is biologically active and its conversion products are capable of undermining DREADD effects.

Full text at www.sciencemag.org/cgi/content/full/337/6095/646-b

Response to Comment on "Impaired Respiratory and Body Temperature Control Upon Acute Serotonergic Neuron Inhibition"

Susan M. Dymecki, Russell S. Ray, Rachael D. Brust, Andrea E. Corcoran, Jun Chul Kim, George B. Richerson, Eugene Nattie

Löffler *et al.* highlight the important potential of designer receptors exclusively activated by a designer drug (DREADD)-based technologies to study cell type-specific functions but cautions that the triggering DREADD ligand, clozapine-*N*-oxide (CNO) might, through potential conversion products, have bioactivity outside of the synthetic DREADD receptor system and maintains that Ray *et al.* did not control for such activity. We recount controls used in our work that indicated no discernible DREADD-independent effects of CNO on the homeostatic assays employed and discuss in this regard murine studies reporting CNO bioneurality in other assays, the rapid renal clearance of *N*-oxides like CNO, and evidence of negligible conversion to clozapine (CN) in mice and rats.

Full text at www.sciencemag.org/cgi/content/full/337/6095/646-c

LOCATION: Jackson
Park Health Club

ARTICLE: *An Electronic
Second Skin*

DATE: Sep 21, 7:43am

LOCATION: Hemlock Bar

ARTICLE: *Quantum
Simulation of Frustrated
Classical Magnetism in
Triangular Optical Lattices*

DATE: Sep 21, 9:21pm

LOCATION: Gyro King

ARTICLE: *Cavemen
Craved Carbs, Too*

DATE: Sep 21, 1:13pm

LOCATION: University
Faculty Lounge

ARTICLE: *The Visual
Impact of Gossip*

DATE: Sep 21, 4:22pm

LOCATION: Bed

ARTICLE: *Consciousness:
What, How and Why*

DATE: Sep 21, 10:56pm



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Comment on “Impaired Respiratory and Body Temperature Control Upon Acute Serotonergic Neuron Inhibition”

Stefan Löffler,^{1*} Jochen Körber,² Udo Nubbemeyer,² Karin Fehsel³

Ray *et al.* (Reports, 29 July 2011, p. 637) assume that clozapine-*N*-oxide (CNO) represents a “biologically inert synthetic ligand” that selectively activates the M4 muscarinic receptor-based DREADD (designer receptor exclusively activated by a designer drug). In contrast, due to the redox cycling of CNO with clozapine and to their cell membrane permeability, CNO is biologically active and its conversion products are capable of undermining DREADD effects.

The DREADD (designer receptor exclusively activated by a designer drug) technology represents a new method with an enormous potential to study cell-type-specific signaling in vivo. The designer receptor here consists of a modified human muscarinic receptor that is activated by clozapine-*N*-oxide (CNO) instead of acetylcholine.

Ray *et al.* (1) assume that CNO represents a “biologically inert synthetic ligand” that selectively activates the inhibitory designer receptor (Di) despite several contrary observations (2–5). Indeed, inertness and specificity of CNO are essential requirements not only for the DREADD method but also for the applied transgenic mouse model that should have been controlled by Ray and co-workers (1).

This comment addresses various biological effects of the redox partners of CNO and of derived products that may interfere with the results of Ray *et al.* (1). All points to which we object here were neither controlled nor discussed by the authors.

N-oxidation of tertiary amines like clozapine (CN) and subsequent reduction back to the corresponding base have been established as common routes of biotransformation (6). In mammals, about 40% of the injected CNO, which is not renally eliminated, is rapidly enzymatically as well as nonenzymatically reduced back to CN (3, 4), putatively accompanied by generation of reactive oxygen species (ROS) (Table 1). Various, partly overlapping, tertiary amine *N*-oxide reduction routes were observed in rodents, including mice (4). Accordingly, mice reduce CNO-like 4-methylpiperazinyl-*N*-oxides of antipsychotic agents efficiently back to their parent compounds (7).

In NMRI mice, intravenous injection of either [¹¹C]CN or [¹¹C]CNO revealed that both pene-

trated the blood-brain barrier within 10 min, but the maximal brain uptake was 4 to 24 times as high for [¹¹C]CN (8). Ray *et al.* (1) injected CNO as a single dose, which initially should lead to about 40% reduction of CNO to CN (2, 7).

In human liver in vitro, CNO is retroreduced to CN in the presence of the reduced form of nicotinamide adenine dinucleotide phosphate. This conversion is inhibited by ascorbic acid (3). CN is metabolized mainly to *N*-desmethyloclozapine (NDMC) (3), a discrete drug with a unique receptor-

binding profile (9), including wild-type M4mAChR (5). NDMC interacts with the *N*-methylscopolamine binding site of all M receptor subtypes, albeit with lower affinity than CN, and is the only clozapine-like compound with partial agonism at the M1 receptor (9). Moreover, all “clozapine-like compounds”—CNO, CN, and NDMC—represent efficacious and potent muscarinic M4 DREADD receptor agonists, with concentration-response curve potency (pEC₅₀) and maximal agonist response (*E*_{max}) values being highest for CN in agonist-stimulated phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 (5). Ray *et al.* (1) did not control for exclusive binding of CNO in the presence of CN, NDMC, and derivatives, especially in the brain, where at least the lipophilic CN transiently accumulates (2, 8, 9).

The quantitative reduction of CNO to CN opens the broad field of receptor-mediated, as well as intracellular signaling pathways of CN and of NDMC. CN, as well as NDMC, binds to a huge number of neuroreceptors, thereby influencing not only the muscarinic but also the dopaminergic, adrenergic, histaminergic, and serotonergic system. Experimental studies with rats revealed a CN-induced hypothermia because of its dopaminergic properties either as D1 receptor agonist (10), D3 receptor agonist (11), or 5-hydroxytryptamine (5-HT) receptor antagonist (12). Hypothermia is

Table 1. Additional CNO effects based on CNO-CN redox cycling, probably confounding the results of Ray *et al.* (1). mPFC, medial prefrontal cortex; GSK3β, glycogen synthase kinase 3β; GABA, (gamma)-aminobutyric acid; GAD67, glutamic acid decarboxylase isoform of 67 kD; Kir3, G protein-gated inwardly rectifying K(+) channel 3; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; LXR, liver X receptor; AhR, aryl hydrocarbon receptor.

Effectors	Targets	Effects	References
<i>Receptor-mediated</i>			
CNO/CN/NDMC	hM ₄ Di (M ₄)	All effectors bind hM ₄ Di	(5, 9)
CN	D3 receptor	Increases in Fos-like immunoreactivity in striatum	(16)
CN	β-Adrenoceptors	Dopamine/noradrenaline release in mPFC	(17)
CN	5-HT receptors	Inhibition of GSK3β, inhibition of respiratory long-term facilitation	(18, 19)
<i>Intracellular</i>			
CN	GABAergic gene promoters (i.e., reelin and GAD67)	Epigenetic DNA demethylation, chromatin remodeling	(20)
CN>NDMC>>CNO	Arrestins	Influence on G(i/o) protein activation	(21–23)
CN	Kir3	Inhibition of CNO-mediated Kir3 activation undermines neuronal silencing by DREADD technology	(14, 24)
CN	PPAR; SREBP; LXR	Immediate disruption of glucose and lipid metabolism	(15, 25, 26)
CN/CNO	AhR	Xenobiotic response; cell differentiation; inflammation	(27, 28)
CN	Mitochondrial and other proteins	Oxidative stress; inhibition of cell respiration	(29–33)

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also a known side effect in CN-treated schizophrenic patients. Besides CN-induced hypothermia, Monda *et al.* (12) studied sympathetic nerve activity in rats. Thirty to 60 minutes after CN injection, the sympathetic firing rate decreased to 50 to 60%, exactly the level reached by DREADD technique in Ray *et al.* (1).

We suppose that the hypothermia of the Di mice is triggered by CN/CNO/NDMC-mediated DREADD effects, as well as by CN/CNO/NDMC-mediated dopamine receptor signaling (10, 11) and 5-HT receptor antagonism (12). In all four trials, a decrease of core body temperature in the control siblings shortly after CNO injection [figure 4A in (1)] may reflect the dopaminergic agonism (10, 11) as well as the serotonergic antagonism of CN (12) and is comparable to the 1 to 1.5°C decrease in body temperature observed previously after CN injection (10, 12). Decisively, the decreased body temperature in the control siblings is induced by CN, not by CNO (10), and thereby confirms CNO retroreduction in the Di mice. Unfortunately, Ray *et al.* (1) did not report body-temperature profiles of the animals before CNO injection [figure 4 in (1)]. Admittedly, the direct CN effect is not as spectacular as the DREADD effect, but CN/NDMC accumulate in the body and may participate in the unexplained severity adaptation [figure 4A in (1)]. In addition, Ray *et al.* (1) omitted a detailed consideration about changes in behavior and physiological factors, which are important in thermogenesis.

Besides the extracellular receptor-binding properties of CN and its metabolites, they are all cell membrane permeable (13) and brain penetrant (8, 9). Focusing on Ray *et al.* (1), proposed targets of the clozapine-like compounds, their effects, and resulting confounders are summarized in Table 1. Among these listed effects, the inhibition of Kir3 by CN is probably most relevant, because neuronal silencing relies on Gi protein-coupled muscarinic

subtype 4 designer receptor (hM₄Di)-mediated Kir3 activation (14).

Although many intracellular CN effects take hours, and the spontaneous reverse reduction of CNO may be negligible in short-term bioassays lasting only minutes, Houseknecht *et al.* (15) and others found immediate perturbations of several metabolic pathways such as hyperglycemia and dyslipidemia after a single parenteral administration of CN. Instead, Ray *et al.* (1) attributed all effects—even the longer-lasting ones (>30 min) or the effects in vivo, which include the hepatic first-pass effect—exclusively to the CNO-Di receptor signaling. They did not control any biological or pharmacological parameters concerning Di receptor-independent CNO/CN effects, although CN itself—without DREADD technology—is able either to modulate or to mimic all the effects observed by the authors, not to mention additional effects triggered by dirty conversion products of CN (3).

In conclusion, scientists working with CNO receptor tools should be on the alert for the redox cycling of CNO as well as for CNO-derived products—CN, NDMC, further metabolites, and ROS/oxidative stress—that are all capable of undermining the designer receptor-based cell and effect specificity.

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Response to Comment on “Impaired Respiratory and Body Temperature Control Upon Acute Serotonergic Neuron Inhibition”

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Löffler *et al.* highlight the important potential of designer receptors exclusively activated by a designer drug (DREADD)-based technologies to study cell type-specific functions but cautions that the triggering DREADD ligand, clozapine-*N*-oxide (CNO) might, through potential conversion products, have bioactivity outside of the synthetic DREADD receptor system and maintains that Ray *et al.* did not control for such activity. We recount controls used in our work that indicated no discernible DREADD-independent effects of CNO on the homeostatic assays employed and discuss in this regard murine studies reporting CNO bioneutrality in other assays, the rapid renal clearance of *N*-oxides like CNO, and evidence of negligible conversion to clozapine (CN) in mice and rats.

In a 2011 study (*1*), we introduced a new Cre-dependent genetic tool, *RC::PDi*, and its parental intersectional (Cre- and Flp-dependent) allele, *RC::FPDi*, for targeting the inhibitory designer receptor exclusively activated by a designer drug (DREADD) (Di) technology (*2, 3*) to highly resolved cell populations in the mouse. We used this tool to determine involvement of serotonergic neurons in physiological homeostasis. Löffler *et al.* (*4*) comment on the important potential of such DREADD-based technologies, yet caution about a potential for CNO to have bioactivity outside the synthetic Di receptor system, in part through potential retroreduction of CNO to clozapine (CN) and/or CN to *N*-desmethylozapine (NDMC). Löffler maintains that Ray *et al.* did not control for Di-independent effects by CNO and its potential conversion products in the reported experiments. For clarification of this point, we emphasize here the critical CNO controls performed and how these controls indicate negligible Di-independent bioactivity of CNO or its potential derivatives in the assays employed. We also discuss work indicating rapid renal clearance of *N*-oxides (e.g., CNO) as compared with tertiary amines (e.g., CN) (*5, 6*) and the negligible back-conversion of CNO to CN in mice and rats (*7, 8*). Retroconversion may be held in check in mice by high tissue levels of ascorbate, an inhibitor of CNO retroreduction

(*7, 9*) that is endogenously synthesized in mice (*10*); by contrast, humans and guinea pigs [the subjects of studies (*8*) cited by Löffler *et al.* as evidence for conversion of CNO to CN in mammals] have lost the capacity to synthesize ascorbate, rely on dietary intake, and typically have lower tissue levels and thus perhaps greater capacity for retroconversion (*11*).

Di is an engineered G_{i/o} protein-coupled receptor (*3*) that, upon binding of CNO, can trigger cell-autonomous hyperpolarization and diminished cell excitability (*2*). We developed a Cre-dependent allele *RC::PDi* and partnered it with the Cre transgenic, *Slc6a4-cre* (or *RC::FDi* with *Pet1::Flpe*) to drive Di expression in serotonergic neurons (*1*). We established that this system offers specific CNO/Di-dependent suppression of serotonergic neuron action potential firing and found that respiratory and body-temperature control was impaired dramatically in a Di-dependent fashion after a single CNO intraperitoneal injection, but not in CNO-treated control mice (non-Di-expressing single transgenic *Slc6a4-cre* or *RC::PDi* siblings).

In each experiment performed by Ray *et al.* (*1*), the effects of CNO administration alone were examined as controls for the experimental conditions, in contrast to Löffler *et al.*'s statement that CNO controls were not performed or discussed. In all experiments, non-Di-expressing single transgenic (*RC::PDi* or *Slc6a4-cre*) sibling controls were assessed for the pertinent physiological parameters before and after CNO injection. For example, in assessing the potential for CNO alone or its metabolites to affect the central respiratory CO₂ chemoreflex [figure 3C in (*1*)], we measured minute ventilation in room air and then in 5% CO₂ before CNO injection, and then again in the same animals after injection during sys-

temic CNO exposure. Respiratory responses pre-CNO and during CNO exposure were indistinguishable in these control animals, showing a normal doubling of ventilation in response to 5% CO₂ as compared with room air. We concluded that in this assay, CNO (or any conversion products) had no appreciable effect on the respiratory CO₂ chemoreflex as measured. Further, ventilatory responses of control animals (with and without CNO) were also indistinguishable from the pre-CNO response of the experimental Di-expressing mice (double-transgenic *Slc6a4-cre, RC::PDi* mice), indicating that Di expression alone (without the triggering ligand CNO) also did not affect the respiratory CO₂ chemoreflex. It was only the combination of Di expression and CNO administration that resulted in an attenuated respiratory reflex [figure 3B in (*1*)].

Similar neutrality of CNO alone or Di alone was observed with respect to body-temperature regulation. While housed at an ambient room temperature of ~23°C, control single-transgenic non-Di-expressing siblings had an average body temperature of 37.9° ± 0.2°C before CNO administration [figure 4 and supporting online material for (*1*)] and an average maximal low of 36.7° ± 0.3°C 2 hours after the CNO injection, indicating an average maximal change of ~1.2°C from the pre-injection baseline. Such ~1°C fluctuations occurred repeatedly in control mice throughout the course of the 20-hour assay and fall within reported normal variation for wild-type mice and healthy humans, including changes associated with handling and subsequent acclimation during temperature measurements (*12, 13*). Also exhibiting body temperatures within the normal range were double-transgenic Di-expressing experimental animals (*Slc6a4-cre, RC::PDi* mice) before CNO administration (36.9° ± 0.2°C). By contrast, extreme hypothermia ensued after CNO administration to these Di-expressing experimental mice, with body temperatures plummeting to 27.1° ± 0.9°C after CNO injection, an ~10°C drop, while being maintained at an ambient room temperature of ~23°C. Given that this extreme hypothermia was specific to Di-expressing mice after CNO administration and was not exhibited by sibling controls in response to CNO, we concluded that Di-mediated effects produced the extreme hypothermia.

Although Löffler *et al.* concur that this extreme body-temperature drop is Di-dependent, he comments that the body-temperature variation of ~1.2°C observed in control animals after handling, CNO injection, and temperature probing is a decisive indication that retroreduction of CNO to CN has occurred. In this regard, Löffler *et al.* cite the work by Salmi and Ahlenius (*14*) and Monda *et al.* (*15*) that shows a temperature drop of up to ~3°C (38.5 to 35.5°C and 37.5 to 36°C, respectively) after direct CN injection in rats. Yet, Salmi and Ahlenius (*14*) also showed in the same article that there is no significant drop in temperature in response to CNO (or NDMC) injection, as compared

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with saline, using doses equivalent to our experiments. Thus, the temperature drop of $\sim 3^{\circ}\text{C}$ cited by Löffler *et al.* (14) is elicited only if rats are directly injected with CN but not CNO, consistent with the negligible CNO effect seen in our assays using mice. Moreover, Guettier *et al.* (7) show no significant retroreduction of CNO to CN in mice over a 2-hour period, as determined by blood plasma liquid chromatography tandem mass spectrometry, and Jann *et al.* (8) report no detectable CN after CNO administration in rats. Additionally, Guettier *et al.* (7) show that by 30 min the plasma concentration of CNO is reduced by half, and by 2 hours it is absent, presumably by renal clearance. Indeed, N-oxides (e.g., CNO), due to their marked polarity, are substantially more soluble than tertiary amines (e.g., CN) and are readily excreted (5, 6).

Löffler *et al.* cite Jann *et al.* (8) as evidence that retroconversion of CNO to CN occurs in mice and rats, yet Jann *et al.* (8) state the opposite, that CN from its N-oxide metabolite CNO was not found to occur in rats, but rather was found in guinea pigs and humans. Thus, not all mammals convert CNO equivalently. Löffler *et al.* note that CNO is retroreduced by human liver microsomal extracts in the presence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and that this retroconversion is inhibited by ascorbate (7, 9). We suggest that inhibition by ascorbate may offer an explanation for the negligible levels of CN detected after

CNO administration in mice and rats. Mice and rats endogenously synthesize ascorbate; humans and guinea pigs have lost this capacity and rely fully on dietary sources. Steady-state liver ascorbate levels are typically 3 to 4 times as high in mice as in humans, varying with age and diet (16–18). These higher ascorbate levels in mice may efficiently inhibit retroconversion of CNO to CN.

In summary, we found that attenuation of the respiratory chemoreflex and extreme hypothermia were dependent on Di expression coupled with CNO administration in double-transgenic *Slc6a4-cre, RC::PDi* mice; these phenotypes were not observed after CNO administration to control Di-negative siblings. CNO neutrality has also been observed in control animals subjected to a host of other physiological and behavioral assays in mice (7, 19–21). We appreciate the opportunity here to recount our experimental design details and, like Löffler *et al.*, we stress the necessity of controlling for effects potentially mediated by CNO alone and/or its metabolites.

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EPIDEMIOLOGY

Preempting Pandemics

Barbara A. Han, Julie Rushmore, Alexa Fritzsche, Dara Satterfield, Jamie Winternitz

You're on the subway when you feel it: the faint tickle of a sore throat, the hint of muscle aches. By dinner-time you've admitted defeat, retreating under the covers with Nyquil and Kleenex. Sick. But this isn't just a bout of seasonal flu. You are one of hundreds of New Yorkers to quietly fall ill in the past 10 days with a novel and highly contagious virus transmitted by rodents. In another month, you will number among millions in a pandemic that has blindsided humanity.

If history is any indication, we are overdue for a plague of this magnitude. Will there ever come a day when we can prevent pandemics altogether? Virologist Nathan Wolfe believes the answer is a resounding yes. In *The Viral Storm*, he sets out to answer three timely questions: "How do pandemics start? Why are we now plagued with so many pandemics? What can we do to prevent pandemics in the future?" Media blitzes about the severe acute respiratory syndrome (SARS) outbreak and swine flu pandemics have captivated the public. In their wake, Wolfe delivers the message that pandemics are imminent threats, a product of our evolutionary history and today's globalized world. Providing vivid accounts of gruesome human deaths, his page-turner carries on the sensationalist spirit commonly surrounding media reports of infectious diseases. According to Wolfe, our best hope for preventing pandemics is to take the offensive, to hunt down deadly pathogens before they have a chance to become front-page news. Proactive surveillance is paramount.

To help readers understand what we are up against, Wolfe introduces "our main character, the microbe" and offers a microbial perspective that stresses humans' position on a planet dominated by viruses and other (often nonpathogenic) microorganisms: "If you were to put on ... magical bug-vision specs, you would instantly see a whole new, and very active, world. The floor would seethe, the walls would throb, and everything would swarm with formerly invisible life." (Germaphobes, you have officially been warned.)

The Viral Storm

The Dawn of a New Pandemic Age

by Nathan Wolfe

Times Books (Holt), New York, 2011. 315 pp. \$26, C\$30. ISBN 9780805091946. Allen Lane, London. Paper, £14.99. ISBN 9781846142987.

Wolfe describes an ongoing evolutionary drama in which humans experience a microbial ebb (e.g., through the advent of cooking) and flow (e.g., through the interconnectedness of our modern world). He singles out viruses as being particularly masterful at keeping pace with continual change, thereby posing a disproportionate threat of pandemic disease. In addition to the biologically and anthropologically informed introductory chapters, Wolfe offers lucid explanations of core concepts from evolution and disease ecology, setting a thorough interdisciplinary foundation for nonexperts.

Between the lines, Wolfe appears as the unmistakable figurehead leading us toward a future free of pandemics. Throughout the book, Wolfe makes his credentials clear. With his top-notch training, international research experiences (in surveillance fieldwork, especially), and collaborations with world-class

scientists, there may be few others poised to bring together researchers from disparate fields in a singular vision to eliminate pandemic threats. Wolfe demonstrates his versatility when discussing the technological advances that make possible his two principal approaches to identifying novel viral threats: The first, microbial surveillance, requires on-the-ground data collected from global disease hotspots. The second, digital surveillance, capitalizes on data from Web-based queries (e.g., a spike in searches for flu symptoms) and cellular text messages (a more common means of communication in remote regions without Internet).

But surveillance is only a first step, and Wolfe forgoes mention of important downstream logistics of confronting pandemics: Coordinating international public health efforts is no simple matter. Vaccine development currently takes over 15 years (1). And traditional responses, such as quarantine or culling reservoir animals, may even exacerbate an epidemic (2). Wolfe's omission of such details in order to deliver a more potent message will undoubtedly rub some scientists the wrong way. However, if the goal is to elevate public literacy about the possibility of preventing pandemics, perhaps this omission is forgivable—scientists are known for burying their message by including too many caveats (3).

A related, pervasive aspect of the book is Wolfe's inconsistency in supplying references to support his sometimes broad-sweeping statements. For example, he mentions the concept of microbial magnification, a process whereby animals high in the food chain harbor a more diverse array of microbes through the accumulation of the microbes of their prey. The absence of supporting references makes it difficult for readers to distinguish whether such concepts are great ideas for future work or have already been evidenced by previous research.

An engaging read, *The Viral Storm* will convince many that eliminating pandemics is possible. But readers may close the book wondering how the many impressive advances in surveillance will translate to the prevention of infectious disease. Let's say we had identified that mysterious rodent-borne virus in New York before it jumped into humans. What course of action would preempt a human outbreak? We are left to imagine for ourselves the answer to Wolfe's final question, "What can we do to



Seeking to stop the spread. A passenger arriving in Hong Kong during the 2003 SARS outbreak wears a face mask to protect himself.

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prevent pandemics?” Nevertheless, the author’s optimistic vision of a pandemic-free future sets the bar for future work. His bold ideas will help motivate the considerable creativity, innovation, and good old-fashioned fieldwork required to reach that elusive pandemic-free calm after the storm.

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WOMEN IN SCIENCE

Improving the Prospects

Kristen Andersen

Women hold approximately 28% of full-time tenure or tenure-track positions in science and engineering fields in the United States, according to data released by the U.S. National Science Foundation (NSF) in 2008. This is substantially lower than the 41% of doctoral degrees in these fields that women received that same year. Although the presence of women in science and engineering has increased over the past several decades, a disproportionately lower number of women pursue upper-level academic positions.

In *Breaking into the Lab*, Sue Rosser seeks to explain why this difference may persist and what might help reduce it. Rosser (a sociologist at San Francisco State University) uses a combination of statistical data, personal experiences (“as a woman scientist and dean at a doctoral research extensive institution”), and interviews with female scientists to suggest possible reasons women have turned away from academia. Her collection of stories illustrates how women have been positively and negatively influenced toward academic careers. Many interviewees noted the importance of a key mentor’s guidance and encouragement and awarding of key opportunities. Often, they found multiple mentors to be

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essential or gained crucial encouragement through networking.

The 50 scientists profiled in the book are past recipients of an NSF Professional Opportunities for Women in Research and Education (POWRE) award or a Clare Boothe Luce (CBL) Professorship. The POWRE awardees, primarily at research institutions, had “received peer-reviewed funding from a focused NSF program in fiscal years 1997–2000.” Most of the five-year CBL recipients had been in their initial tenure-track position at liberal arts colleges. Because the perspectives presented in the book are those of women scientists who successfully pursued upper-level academic positions, the reasons why one might leave academia for other sectors are not fully examined. Nonetheless, the interviews do provide some insight into negative influences that can lead women scientists to choose not to pursue an academic career.

One theory recurring throughout the book suggests that the accumulation of multiple small discriminations and negative experiences turns many women away from academic positions. These micro-inequities build up over time, fueling anger and resentment toward male colleagues. This can exacerbate the woman scientist’s growing unhappiness and deepen the rift between her and her colleagues. She may eventually choose to leave academia for a work environment she deems less hostile. Rosser reminds read-

ers that negative surroundings—at the levels of individual labs, departments, or entire institutions—can greatly hinder a woman scientist’s progress. Her isolation from departmental colleagues or the establishment of negative relationships can quickly make a position unbearable.

The experiences Rosser recounts illustrate that the path to obtaining tenure, and remaining highly productive thereafter, is fraught with obstacles. A lack of family care or difficulties in negotiating a good start-up package may lead women to fall behind male peers in terms of research productivity, thus handicapping them during their tenure evaluation. The interviewees also reported being overextended. In the act of providing diversity, departments and institutions often called on them to serve as the quintessential woman on a committee, thereby requiring extensive service from them. Such demands take time away from research, which generally remains the principal factor in obtaining tenure.

Rosser suggests that women scientists must seek out positive environments, mentors, and supportive networks to ensure success. Although this approach seems straightforward, we must acknowledge that it is often

difficult to foresee issues with a mentor or department until problems begin to accumulate. The book also offers advice for mentors, departments, and institutions hoping to improve the retention and promotion of women scientists—recommending steps such as helping with start-up negotiations and providing special awards

and networking opportunities. Of course, various institutions and organizations have already acted along these lines. Groups such as the Association for Women in Science and programs such as the NSF ADVANCE initiatives focus on facilitating connections between women and other key aspects for career advancement.

The extent of the solutions proposed, however, calls into question whether it is necessary to make special adjustments for women. Many of the issues addressed by Rosser could apply to both men and women, even if, as Rosser suggests, women take negativity harder. Despite the hurdles, many women scientists have attained high-level positions in top-tier institutions without special treatment. An effective and just solution requires a fine balance between promoting women in science and the establishment of rules and policies that favor neither gender.

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Breaking into the Lab Engineering Progress for Women in Science

by Sue V. Rosser

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New York, 2012. 261 pp. \$35.
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WATER MANAGEMENT

Water Sustainability for China and Beyond

Jianguo Liu* and Wu Yang

A water crisis has prompted the Chinese government to develop an ambitious water conservancy plan. However, the plan may not achieve water sustainability and may cause unintended environmental and socioeconomic consequences, unless it accounts for complex human-nature interactions (1). Water shortages, for example, force people to find alternatives, such as treatment facilities, whose land and energy requirements aggravate food and energy production, which need large amounts of water. Other nations face similar challenges and share real water from China along international rivers and/or virtual water through trade. Water problems are particularly challenging in China, which has the largest population, fastest-growing economy, rising water demand, relatively scarce water, dated infrastructure, and inadequate governance. We highlight China's water crisis and plan, and then offer recommendations.

Water Crisis and Grand Investment

Two-thirds of China's 669 cities have water shortages, more than 40% of its rivers are severely polluted, 80% of its lakes suffer from eutrophication, and about 300 million rural residents lack access to safe drinking water (2). China's per capita availability of renewable water resources is about a quarter of the world average, but water consumption per unit of Gross Domestic Product is three times the world average because of water-intensive industrial structure, outdated technologies, low reuse rate, and wastefulness (2). Uneven natural water distribution aggravates the crisis. Influenced by a monsoonal climate, precipitation generally declines from the southeast to the northwest and varies greatly from year to year and season to season (2). Water-related disasters such as 1998 floods and 2010 droughts saw large losses of life and property, as well as damage to human well-being and the environment.

In January 2011, the government's annual "No. 1 Document," which reflects its top pri-

orities, outlined a plan to expedite water conservancy development and reform and to achieve sustainable use and management of water resources within this decade (see the supplementary materials). The plan is to quadruple total investment in solving water problems to four trillion yuan (U.S. \$635 billion) in the next 10 years compared with the investment in the past decade (see the graph).

More than 46,000 of the 87,000 dams and reservoirs built since the 1950s have surpassed their life spans, or will within 10 years, which will increase the risk of structural failure (3). The central government plans to repair and reinforce them by the end of 2015 (3) and also build new dams, reservoirs, and canals. Since the 1950s, China has constructed 12 major projects that annually divert more than 9 billion m³ of water; many more such projects are being constructed or planned (table S1), including the South-North Water Transfer Scheme—the world's longest and largest water diversion project with a planned investment of 486 billion yuan (U.S. \$77 billion) and 45 billion m³ of annual water transfer.

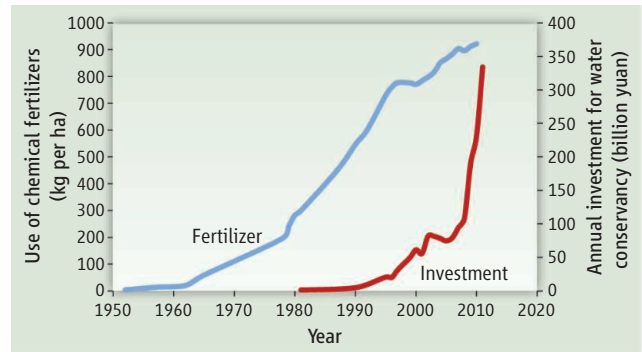
The plan focuses largely on engineering measures related to water quantity. Except for water-related targets in the 12th Five-Year Plan, the water plan does not set quantitative targets for water quality, which is low in China and severely affects human and ecosystem health [e.g., increased cancer mortality is attributed to water pollution (4)].

Policy Recommendations

This planned investment is laudable, but not sufficient. Thus, we offer the following.

Coordination. Many agencies are involved in water governance (table S2) but lack coordination. At the central government level, the Ministry of Water Resources (MWR) leads design and implementation of the new water plan. The Ministry of Environmental Protection (MEP) is supposed to be in charge of water pollution prevention and control, but

Despite investments in water infrastructure, China must address complex human-nature interactions to ensure supply and quality.



China's investment in water conservancy (19–21) and use of chemical fertilizers (22).

lacks adequate resources and jurisdiction. Many water projects (e.g., Jinsha River hydropower) were rushed without following the national law of environmental impact assessments and have caused enormous environmental and socioeconomic impacts (5). New laws are needed regarding development (e.g., hydropower) of rivers and river basins (5). To implement laws, relevant sectors and agencies (e.g., water resources, environmental protection, shipping, and agriculture) must coordinate and punish law-breaking behaviors.

Coordination must cross organizational levels and sectors. Many water-related laws and policies are not implemented because central and local governments' efforts are not coordinated. In 2004, the central government promulgated a policy to stop construction of golf courses, which consume and pollute much water. Yet over 400 golf courses have since been built (6). The government encourages urbanization, but water protection does not receive as much attention as energy (e.g., green building codes tend to focus more on energy and less on water). The number of households has been growing more rapidly than population due partly to increased divorce and shrinking of multigenerational families (7). Rapid housing and land development consumes much water, generates wastewater, and expands impermeable surfaces that increase runoff (8).

China has achieved remarkable food production, in part through expansion of irrigation and use of pesticides and chemical fertilizers (see the graph), which has escalated water pollution and depleted surface- and

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groundwater. More water will be needed by mines as China expands coal production (9). Water diversion projects may encourage consumption and increase demand. The Luanhe-to-Tianjin project annually diverted roughly one billion m³ of water into Tianjin (table S1), but Tianjin's water-fueled growth now demands even more from the South-North Water Transfer Scheme. China's plan aims to reduce water use in irrigated agriculture (61% of total water use in 2010) largely by constructing efficient farm infrastructure. This may actually increase water use and redistribute supply at a larger scale (e.g., water basin), reducing aquifer replenishment and intensifying depletion at large scales (10).

Although water demand is growing, supply is increasingly limited. Glaciers are retreating due to climate change (11), reducing water supply. Depletion of water reduces biodiversity (thus, ecosystem services such as water purification) and causes pollution such as eutrophication. Pollution and the spread of invasive species through water diversion may compromise water quality and supply.

Coordination requires continued efforts to lower population growth and slow household proliferation; clarify water rights (e.g., setting maximum water-use quotas at various levels and for all entities); reform water pricing (e.g., increasing block tariffs that discourage high usage); and eliminate inefficient practices (e.g., through rebates for water-saving equipment). More secure land rights to farmers may encourage them to sustain their lands by avoiding overuse of fertilizers and thus protect water. Incentive programs alone cannot guarantee less water use (10). Both "hard path" (e.g., dams) and "soft path" (e.g., public education, efficient technology, and financial incentives) approaches are needed (12).

Institutional innovations are indispensable. An effective entity (e.g., a sustainability coordination office) is needed directly under the State Council (the country's highest executive organ) to clarify functions and responsibilities of water management agencies (table S2), reduce institutional mismatches, identify shared interests, avoid conflicts, and bridge gaps. With the State Council's support, it would have resources and authority to work across agencies and sectors, as well as with the public and non-governmental organizations. It also would facilitate public communication and disclosure of information to make government transparent and accountable.

Integrated monitoring and proactive measures. Current monitoring programs include indicators such as water quantity and use efficiency (by the MWR) and water quality (by

the MEP). To more accurately predict changes and take proactive adaptive management measures, it is essential to monitor indicators that drive changes in water quantity and quality directly and indirectly. Ministries should expand monitoring, particularly in areas with severe water shortages, to indicators in human dimensions (e.g., values and attitudes toward water, land use, and development).

To minimize negative legacy effects and avoid irreversible impacts, China can learn from other countries (e.g., the U.S. Clean Water Act). China should take proactive measures (e.g., solicit public opinions before implementation) for major projects; track government officials' responsibilities and performance related to water; and expand the major evaluation and promotion criteria of short-term economic performance.

Integrating social sciences. China has been vigorously promoting natural sciences and technology but lags behind in integrating them with social sciences, development of which was constrained for decades by government's political ideology (13). Incorporating social sciences is not a panacea, but can help, e.g., by predicting water demand and long-term effects of China's water plan, and could lead to positive changes in human behaviors. Efficiency of the plan can be enhanced through changing people's values, attitudes, and behaviors toward water. Changing consumption is crucial for water sustainability, because engineering measures (e.g., long-distance water transfer) have caused serious socioeconomic and environmental costs, despite enormous benefits (12).

Enhancing international cooperation. China's water plan does not consider virtual water in trade or real water in international rivers. China's trade during 1996–2005 caused a net loss of 23.5 billion m³ of virtual water (14). Although virtual water is but one consideration in trade, and there may be other benefits and costs, it is important for achieving water sustainability. Use of real water in international rivers also has broad implications. Headwaters of 12 major international rivers are in China (15), and conflicts have increased over their use in recent years (16).

It is important for China to take a more active role to reduce its water footprint and to cooperate on the use of international rivers. For example, although the Mekong River Commission (MRC) has been operating since 1995, its effectiveness may be improved by including China. China has been uninterested because joining MRC would compromise sovereign control and limit unilateral action. Thailand was reluctant to admit China because it did not want MRC to be dominated

by communist countries (China, Lao PDR, and Vietnam) (17). Lessons can be learned from other international cooperative efforts (e.g., the Nile Basin Initiative).

Conclusion

Policies for water and other issues should be jointly designed, implemented, monitored, and evaluated. Integrated Water Resource Management (IWRM) may be a useful approach. China has begun to adopt IWRM concepts, although it is not easy (18). But China now has financial resources and motivations to address the water crisis and to generate lessons for achieving sustainability in China and the rest of the world.

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Supplementary Materials

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VIROLOGY

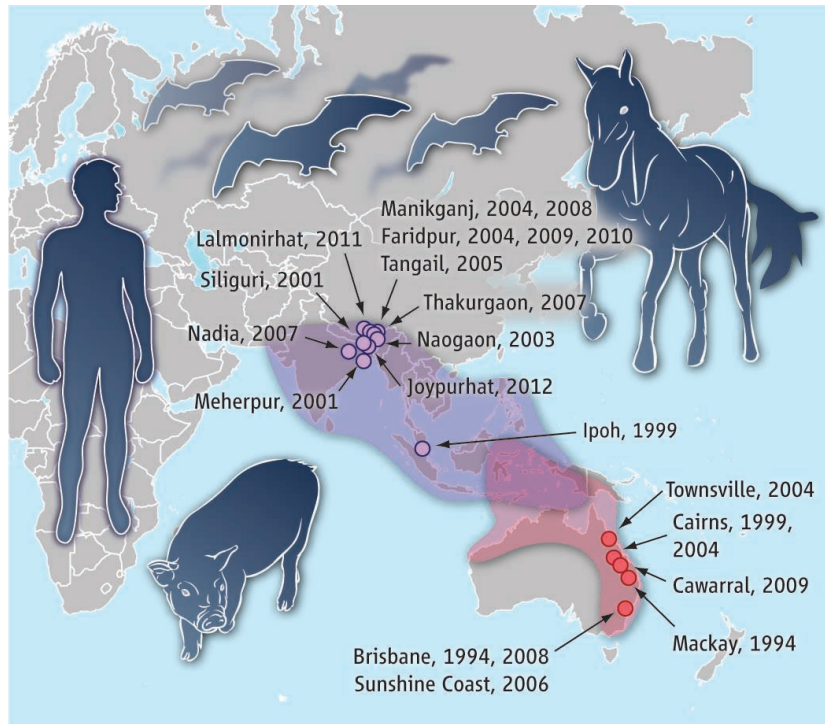
A Henipavirus Vaccine in Sight

Veronika von Messling¹ and Roberto Cattaneo²

Nipah virus emerged in pig farms in Malaysia in the 1990s. Outbreaks were difficult to miss: The cough of infected animals could be heard 1 mile away from the farms. It soon became clear that Nipah is an important zoonotic threat. Fruit bats can transmit the virus to many mammals, including humans (1). The frequency of recurring Nipah virus outbreaks is on the rise (2). These outbreaks, which often affect Bangladesh or India, have caused more than 300 human fatalities since 1999 often due to encephalitis.

A related virus named Hendra emerged in 1994 in Australia. Also carried by bats, Hendra causes fatal disease, especially in humans and horses (3). Together, Hendra and Nipah form the new *Henipavirus* genus (2) of the Paramyxoviridae, a family of negative-strand RNA viruses that includes measles, mumps, and human respiratory syncytial virus. Bossart *et al.* now show that a vaccine based on a soluble form of the Hendra virus glycoprotein can protect monkeys from potentially lethal challenge with the Nipah virus (4). The opportunity to prevent or at least reduce human suffering and economic losses, and to maintain social peace, is tremendous.

Many of the Southeast Asian countries affected by Nipah outbreaks (see the figure) are growing economies with young and mobile populations. The associated spreading urbanization has led to the destruction of previously untouched wilderness, and lifestyle changes are driving the transition from traditional to more industrial agriculture, including high-density pig and poultry production. The resulting increased frequency of contact among wildlife, domestic ani-



Outbreak. Recent and recurring outbreaks of Nipah (blue) and Hendra (red) viruses in human, bat, pig, and horse have been observed in Southeast Asia, India, and Australia (3, 15).

mals, and people facilitated the emergence of several previously unknown pathogens. The most visible is H5N1 avian influenza, which elicited global pandemic preparedness efforts after the first human cases were reported in Hong Kong in 1997 (5). In addition to leading to new processes for accelerated vaccine and drug approval, these efforts have stimulated the development of regional public health agendas and increased awareness of the human-animal interface.

In contrast to H5N1 influenza, most emerging pathogens—including Hendra and Nipah viruses—are still considered minor problems, limiting the resources available for the development and subsequent regulatory approval of vaccine candidates or antiviral drugs. Ongoing collaborative efforts of international groups have now yielded a safe and broadly protective subunit vaccine; different formulations of the recombinant subunit (developed through DNA techniques) are effective in protecting ferrets (6) from lethal Hendra challenge, or protecting cats (7) and African green monkeys (4) from lethal Nipah challenge. The fact that the lim-

A candidate vaccine against Nipah and Hendra viruses, recently emerging zoonotic threats, looks promising.

ited market may not attract large pharmaceutical companies provides a unique opportunity for local leadership in vaccine development.

Producing the vaccine first for veterinary use, as planned in Australia for Hendra protection (8), may reduce infection in domestic animals and thereby the spread to humans. Such a subunit vaccine may be a primer for an integrated approach to control airborne zoonotic diseases by targeting the animal reservoir, emulating strategies successfully used to reduce the disease burden associated

with food-borne pathogens such as *Salmonella* or *Escherichia coli* (9). Nonetheless there is a continued need for a vaccine that is approved for use in humans; horses can transmit Hendra virus to humans, whereas bats, pigs, and even humans can transmit Nipah to humans (10).

The development of a veterinary vaccine should generate information on the duration and level of protection against Hendra and Nipah viruses. Analogously, vaccination efficacy against rabies, influenza, and West Nile virus is routinely documented in farm animals, at a fraction of the costs associated with human clinical trials. These data, and additional experimental vaccine-challenge studies in monkeys, could provide a starting point for prospective clinical trials. These trials could benefit high-risk regions (such as Bangladesh or parts of Malaysia) and could include individuals with high risk of exposure, such as horse farmers or veterinarians.

Because of the history of exacerbated disease in children immunized with formalin-inactivated Paramyxovirus such as the measles or respiratory syncytial virus prepa-

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rations (11, 12), vaccines against other Paramyxoviruses based on inactivated virus raise immediate concerns. Although the Bossart *et al.* study gives no indication that similar issues may arise with the Hendra virus glycoprotein subunit vaccine, the duration of protection, and the course of disease in individuals with waning antibody titers, will have to be carefully investigated.

In any case, the parallel development of a vaccine based on a live attenuated virus is prudent because, in analogy with the extremely safe measles vaccine (13), it could have a longer-lasting protective effect. Notably, numerous Paramyxovirus-like sequences were recently documented

in bats (14), raising the specter of the existence of a huge reservoir of emerging Henipaviruses and further motivating vaccine development.

Once production processes yielding a stable Henipavirus recombinant subunit vaccine are established, the crucial next step will be its rapid distribution to contain or at least slow down contagion. Viruses do not respect borders, and distribution will have to rely on efficient international networks. Thus, Henipa vaccine distribution is strong motivation for establishing international networks that unite medical, veterinary, and public health communities to reduce the global impact of infectious diseases.

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CHEMISTRY

Uncovering the Uranium-Nitrogen Triple Bond

Alfred P. Sattelberger and Marc J. A. Johnson

Transition metal complexes containing multiple bonds to a carbon, nitrogen, or oxygen ligand (L) play a critical role in a wide range of important chemical and biological transformations (1). The chemistry of the actinide element uranium (U) has certain similarities to that of molybdenum and tungsten, and inorganic chemists have sought to expand uranium's multiple-bond chemistry beyond that of the uranyl functional group, UO_2^{2+} , for some time. One of the more elusive targets has been a uranium compound with a terminal or "naked" $\text{U}\equiv\text{N}$ group—a nitrido ligand in which the N atom has no bridging interactions. Several research groups (2–7) have tried to isolate such a molecule but were thwarted by the high nucleophilicity and associated reactivity of the terminal $\text{U}\equiv\text{N}$ moiety. On page 717 of this issue, King *et al.* (8) report having isolated and thoroughly characterized a high-valent U^{V} complex with a terminal nitride ligand using an elegant synthetic strategy.

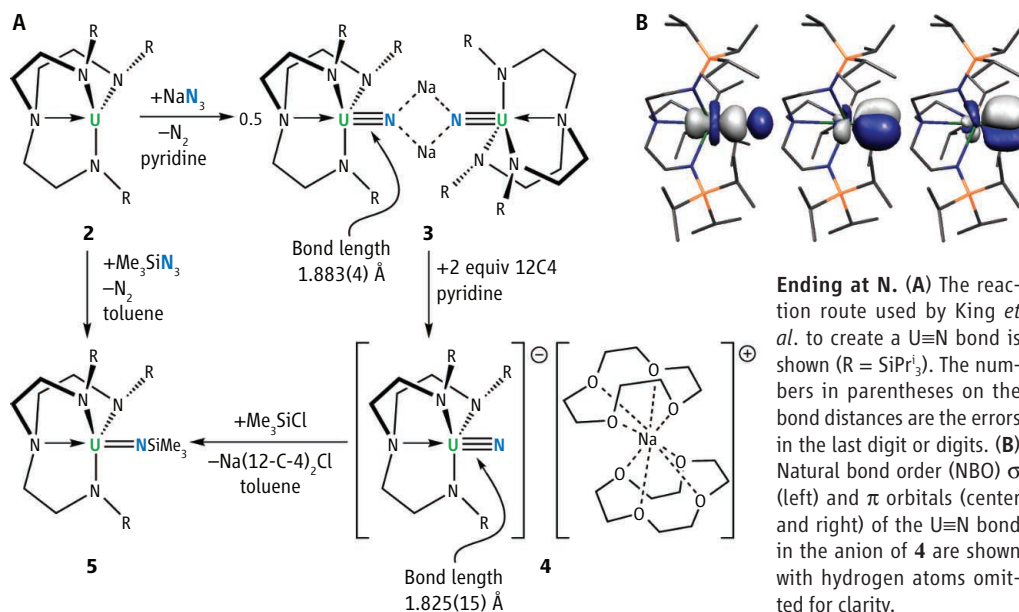
King *et al.* reasoned that isolation of a molecule of the type $\text{L}_n\text{U}\equiv\text{N}$ would require an excep-

tionally crowded ligand environment around the U center, one that would preclude intra- and intermolecular decomposition pathways. They designed and synthesized the sterically demanding trianionic ligand $\{\text{N}(\text{CH}_2\text{CH}_2\text{NSiPr}_3)_3\}^{3-}$ or $\text{Tren}^{\text{TIPS}}$ for short, where Pr^i is isopropyl. They isolated the green U^{IV} compound $(\text{Tren}^{\text{TIPS}})\text{UCl}$ (**1**) via the low-temperature reaction of $\text{Li}_3\text{Tren}^{\text{TIPS}}$ with UCl_4 in tetrahydrofuran, followed by recrystallization of the crude product from

A triple bond formed between a high-valent uranium atom and a nitrogen atom has appreciable f orbital character.

toluene. Potassium metal reduction of **1** in hexane solvents afforded the deep purple U^{III} compound $(\text{Tren}^{\text{TIPS}})\text{U}$ (**2**) in high yield.

The x-ray structure of **2** revealed a deep pocket in which a linear molecule might coordinate. Oxidation of **2** with sodium azide in pyridine (see the figure, panel A) eliminated N_2 and formed the red dinuclear, sodium-bridged U^{V} salt **3**. The x-ray structure of centrosymmetric **3** revealed two U–N groups (with a bond distance of 1.883 Å)



Ending at N. (A) The reaction route used by King *et al.* to create a $\text{U}\equiv\text{N}$ bond is shown ($\text{R} = \text{SiPr}_3$). The numbers in parentheses on the bond distances are the errors in the last digit or digits. (B) Natural bond order (NBO) σ (left) and π orbitals (center and right) of the $\text{U}\equiv\text{N}$ bond in the anion of **4** are shown with hydrogen atoms omitted for clarity.

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bridged by two potentially labile Na^+ cations. Treatment of **3** with two equivalents of the chelating, cyclic ether 12-crown-4 (12C4) encapsulated the Na^+ ions and the red, ion-separated U^{V} salt $[(\text{Tren}^{\text{TIPS}})\text{U}\equiv\text{N}][\text{Na}(12\text{C}4)_2]$ (**4**) was isolated. Although **4** proved very reactive and decomposed rather quickly in ethereal solvents to form intractable products, it reacted readily with trimethylsilyl chloride (Me_3SiCl) in toluene to form the neutral red U^{V} imido complex $(\text{Tren}^{\text{TIPS}})\text{UNSime}_3$ (**5**), a compound that the authors prepared independently by treatment of **2** with trimethylsilyl azide (Me_3SiN_3) in toluene.

The crystal structure of **4** revealed the long-sought terminal $\text{U}\equiv\text{N}$ moiety with an anticipated short U-N distance of 1.825 Å, which is shorter than the 1.916 Å distance found in the Fox and Cummins borane-capped U^{V} nitride compound, $[(\text{C}_6\text{F}_5)_3\text{BNU}(\text{NArBu}^t)_3]^-$ ($\text{Ar} = 3,5\text{-Me}_2\text{C}_6\text{H}_3$) (**9**). The structural results on compounds **3** and **4** are supported by a battery of analyses, including infrared and optical spectroscopy, isotopic labeling with ^{15}N , elemental analyses, and the liberation of ammonia from **4** upon treatment with water. The U^{V} valence assignment is also supported by the temperature-dependent magnetic susceptibility data. Both **3** and **4** are electron paramagnetic resonance silent down to 5 K, as found in some trigonal U^{V} imido complexes (**5**, **10**). The authors calculated the electronic structures of dimer **3** and the free anion of **4**, $[(\text{Tren}^{\text{TIPS}})$

$\text{U}\equiv\text{N}]^-$, using density functional theory. The metrical parameters calculated for the two compounds are in good agreement with the x-ray structural data. The single U 5f electron resides in a nonbonding highest-occupied molecular orbital that is essentially pure 5f in character, which suggests that it is a target for removal (see below). The $\text{U}\equiv\text{N}$ triple bond is a result of population of two quasi-degenerate π orbitals and one σ orbital, with the latter being higher in energy. This orbital ordering is similar to that of the uranyl cation, UO_2^{2+} (**11**).

A natural bond order (NBO) analysis (**12**) of **4** indicates that the σ and π orbitals associated with the $\text{U}\equiv\text{N}$ bond are roughly 30% U and 70% N (see the figure, panel B). The U component of both types of bonds has appreciable 5f orbital character—43% of the σ bond and 72% of the π bonds. The calculated U-N Mayer bond order is 2.91 for **4**, consistent with the triple-bond representation (**13**). It decreases to 2.21 in **3**, presumably as a result of the polarizing influence of the Na^+ cations. The calculated charge on the nitride N in **4** is -1.36 , which is consistent with a highly nucleophilic center and the tight coordination of Na^+ in **3**.

These studies were inspired not only by an interest in fundamental uranium chemistry but also by that of the nuclear science community in uranium nitride, $(\text{UN})_x$, an attractive ceramic fuel for advanced nuclear reactors (**14**). Further elaboration of the chemistry described by King *et al.* could take several

directions. For example, complexes **3** and **4** would appear to be ideal starting materials for further synthetic explorations of high-valent uranium chemistry. It may be possible to oxidize compound **4** to the neutral U^{VI} compound $(\text{Tren}^{\text{TIPS}})\text{U}\equiv\text{N}$ or reduce it to the U^{IV} dianion, $[(\text{Tren}^{\text{TIPS}})\text{U}\equiv\text{N}]^{2-}$. The U^{III} compound **2** is also a candidate for further investigations, as it will likely react with other small molecules like CO, NO, N_2O , and CO_2 .

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CELL BIOLOGY

Precursor or Charge Supplier?

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Phosphorylated derivatives of phosphatidylinositol (PI) play critical roles in cell signaling and in regulating membrane traffic and cytoskeletal architecture (**1**). Phosphatidylinositol 4-phosphate (PI4P), for instance, is essential to the formation of vesicular carriers in the *trans*-Golgi network (**2**), and serves as a substrate for the synthesis of phosphatidylinositol (4,5)-bisphosphate $[\text{PI}(4,5)\text{P}_2]$ in the plasma membrane. On page 727 of this issue, Hammond *et al.* (**3**) reveal an

unappreciated function of plasmalemmal PI4P in controlling protein recruitment and ion channel activity.

Hammond *et al.* show that most plasmalemmal PI4P is not involved in, or required for, $\text{PI}(4,5)\text{P}_2$ synthesis. Instead, their results show that—as reported for $\text{PI}(4,5)\text{P}_2$ —PI4P recruits soluble polycationic proteins to the plasma membrane. Moreover, PI4P also can influence the activation of ion channels. These effects are likely electrostatic, mediated by the contribution of PI4P (which carries two or three negative charges at physiological pH) to the surface charge of the inner leaflet of the plasma membrane (see the figure).

What is the source of the PI4P involved in $\text{PI}(4,5)\text{P}_2$ synthesis? Hammond *et al.* show

A phospholipid directly influences the recruitment and function of proteins at the plasma membrane.

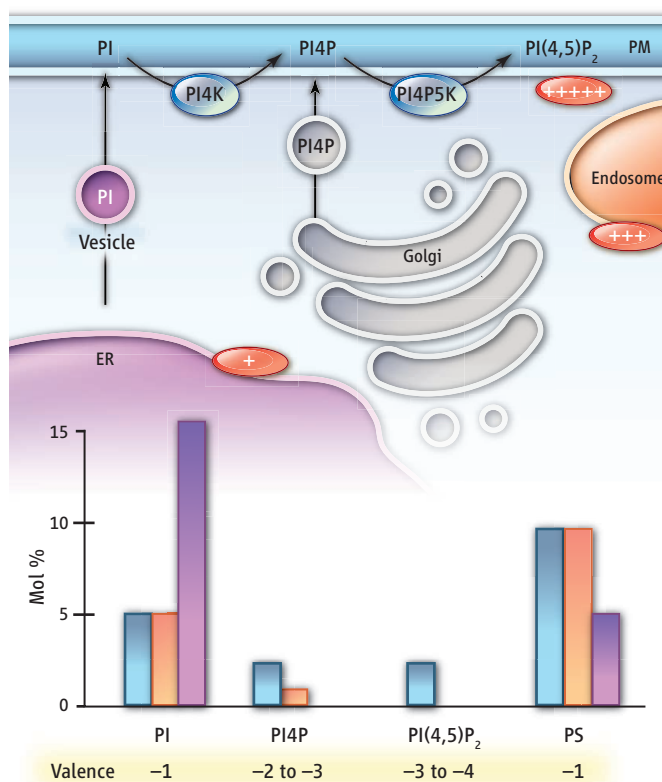
that after depletion of both phosphoinositides, the $\text{PI}(4,5)\text{P}_2$ pool can be replenished with negligible increases in PI4P. It is conceivable that a minor pool of PI4P that is newly formed at the plasma membrane is rapidly converted into $\text{PI}(4,5)\text{P}_2$. A “channeling” process would deliver the PI4P formed by plasmalemmal PI 4-kinase (PI4K) directly to PI4P 5-kinase (PIP5K), thereby accumulating $\text{PI}(4,5)\text{P}_2$ with little change in PI4P, which may thus remain at undetectably low concentrations. Reports of physical association between PI4K and PIP5K (**4**) support this channeling concept. The process may be aided by transfer proteins that present PI to kinases (**5**). PI4P transfer activity has also been described for other lipid transporters (**6**).

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Alternatively, it is possible that PI4P that is phosphorylated by plasmalemmal PIP5K originates from internal sources. PI4P is central to forming vesicles delivered from the trans-Golgi network to the plasma membrane, and their lipid cargo could be the main substrate for plasmalemmal PI(4,5)P₂ synthesis. Another possible source is the endoplasmic reticulum (ER)-derived PI-synthesizing mobile element (7). Although the ER is thought to be largely devoid of PI4P, two classes of PI4Ks have been found in this compartment (8, 9) and are possibly present in the PI-synthesizing mobile element derived therefrom. Although ER-derived vesicles do not directly fuse with the plasma membrane, “kiss-and-run” fusion (in which vesicles transiently fuse with the membrane, without fully merging with it) or soluble lipid transporters could deliver lipids to the plasma membrane. These possibilities should be considered, but it remains unclear whether constitutive secretion of intracellular vesicles can deliver sufficient PI4P to maintain and replenish plasmalemmal PI(4,5)P₂ after acute depletion.

Regardless of its source and contribution to the synthesis of PI(4,5)P₂, Hammond *et al.* identify PI4P as a major contributor to the negative charge of the inner surface of the plasma membrane. This not only locally concentrates cationic species within the electrical double layer but also drives the adsorption of soluble polycationic species onto the inner surface of the membrane. PI4P can also tightly bind to positively charged domains of the cytosolic aspect of transmembrane proteins, altering their conformation and function. This accounts for the modulation of ion channels described by Hammond *et al.* and others.

Negatively charged phospholipids—PI(4,5)P₂, PI4P, PI, and phosphatidylserine (PS)—are more abundant in the inner leaflet of the plasma membrane than in any other cellular membrane. The contribution of PI(4,5)P₂ and of the less charged but highly abundant PS to the inner surface charge had been recognized, but the role of PI4P had not been fully appreciated. Membrane charge is dictated by the relative abundance and valency of the anionic lipids and, to this extent, the contribution of PI4P (which constitutes 1 to 2% of the plasma-



Phosphoinositide distribution and charge dictate organellar identity. PI and PI4P made in the ER and Golgi, respectively, are delivered to the plasma membrane (PM) where they are converted to PI(4,5)P₂ either by independent (uncoupled) PI4P5Ks or by kinases that use PI4P channeled directly by a plasmalemmal PI4K. Organelles have distinct surface charge due to their differing anionic lipid composition (colors in bar graph correspond to organelles). Each organelle can thus differentially recruit soluble polycationic proteins (shown with plus signs) as well as alter the structure of transmembrane proteins with cytosolically exposed cationic domains (not shown).

lemmal lipid and carries a -2 to -3 charge) should have been anticipated. These generic considerations assume that phospholipids are homogeneously distributed, but this presumption is likely naïve and incorrect; the lateral distribution of lipids in the plane of the membrane is probably inhomogeneous, with areas of varying charge density. Indeed, super-resolution and electron microscopy have demonstrated the presence of PI(4,5)P₂- and/or PS-enriched clusters in defined (nano)domains of the membrane, notably in caveolae (10, 11). The submicroscopic arrangement of PI4P in the membrane is not known at present, due in part to a lack of suitable probes. Nevertheless, some of the plasmalemmal PI4P is likely enriched in specialized domains; certain membrane regions might be devoid of PI4P5K, which could explain the existence of PI4P-rich patches and of autonomous PI4P pools.

Unlike PI(4,5)P₂, PI4P (like PS) is present in substantial amounts in endomem-

branes. Although the predicted net surface charge of such organellar membranes would be less than that of the plasma membrane, it is not negligible and likely influences the recruitment of polycationic proteins with more moderate charge. Thus, the particular phospholipid composition of each organelle defines its electrostatic identity and thereby attracts a subset of cationic targets. How this electrostatic identity is established and maintained depends on the interplay of lipid synthases, kinases, and phosphatases, but will also be influenced by both vesicular and nonvesicular transport of lipids.

To date, most attention has focused on the role of PIs as molecular beacons, interacting stereospecifically with proteins that contain modular domains, such as pleckstrin homology, Phox homology, or FYVE, that recognize the head-groups of specific inositides. Less attention has been paid to the electrostatic contribution of phosphoinositides to protein recruitment and retention. Many proteins contain polycationic sequences that should be attracted to negatively charged surfaces. Moreover, structural studies show

highly cationic patches on the surface of several peripheral membrane proteins. This suggests that many functionally important proteins use this conceptually simple, yet fundamental mechanism of membrane targeting. It will be important to keep electrostatic associations in mind when considering the physiology of PI4P and other anionic lipids.

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APPLIED PHYSICS

A Different Angle on Light Communications

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A conventional propagating free-space laser beam typically has an approximately flat phase front and exhibits an intensity profile that decreases as a function of the radial distance from the beam center. However, in the 1990s, it was shown that propagating light waves can contain an interesting property known as orbital angular momentum (OAM) (1). OAM implies that the light wave's phase front is twisting along the direction of propagation. This twisting of the phase front into a corkscrew shape results in a doughnut-like ring intensity profile (2). It was then demonstrated that a given optical beam with OAM can be encoded with data (3–5). Moreover, a radiowave field trial showed that a free-space radio data link can use OAM for trans-

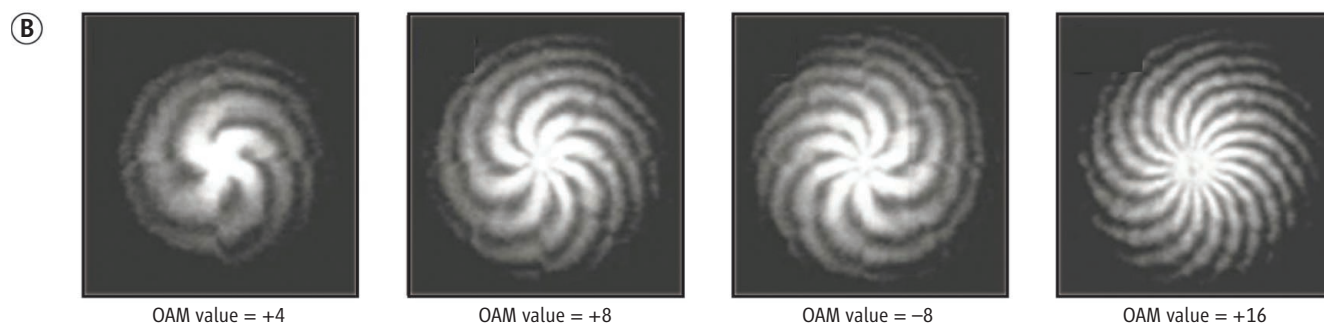
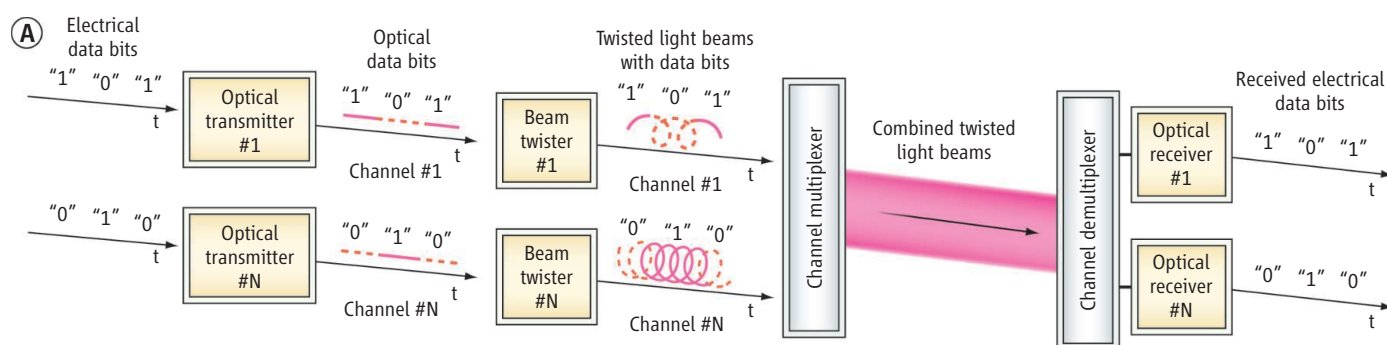
mission (6). Recent work has demonstrated a 2.56 terabits per second (Tbit/s) free-space optical data transfer using OAM (7).

A fascinating aspect of OAM is that an infinite, discrete series of values for the rate of the phase front twisting can be created, such that each OAM beam with one value is theoretically orthogonal to (that is, distinguishable from) all other OAM beams with different values. Orthogonality enables multiple beams of different OAM values to be multiplexed together and subsequently demultiplexed with negligible crosstalk between them. Each of these multiple beams can carry an independent data stream, and the total information capacity of the spatially overlapped beams equals the data rate of one beam multiplied by the total number of independent beams (see the figure) (7). Importantly, each optical beam can reside at the same optical carrier wavelength (color), thereby increasing the communication system's spectral efficiency in terms of the number of data bits per second per Hertz of bandwidth.

Can “twisted” light beams enhance optical communication systems?

Multiple techniques were used to achieve a capacity of 2.56 Tbit/s in the free-space optical transmission link using OAM multiplexing (6). At the transmitter, spatial light modulators (SLMs) were used to create “phase holograms” that would induce a specific twist on a conventional light beam. Subsequently, a different SLM having an inverse phase hologram would undo the twisting at a receiver. Initially, four SLMs induced four different OAM values on four independent beams. Each independent beam was modulated with digital data, and 80 Gbit/s was encoded on the instantaneous amplitude and phase of each of the four original beams; this higher-level modulation format is called 16 quadrature amplitude modulation (QAM) (8). Note that simple on-off-keyed amplitude-modulated data could have been used, but such modulation tends to have a lower capacity and spectral efficiency.

Three different methods that each doubles the number of data-carrying beams were used in succession, thereby creating



Communicating with a twist. (A) Multiple data channels, each on a different light beam having a wavefront that twists in a different helical shape (i.e., OAM values) as it propagates, can be multiplexed together to produce an aggregate

of Tbit/s of free-space data transmission capacity. (B) Measured phase interferograms corresponding to four beams with different OAM values: OAM_{+4} , OAM_{+8} , OAM_{-8} and OAM_{+16} .

32 data streams from the original 4 OAM beams and achieving the overall Tbit/s data capacity. In each method, each initial beam was split into two identical beams, with one beam being modified such that the two are distinguishable from each other, and then recombining the two beams for transmission: (i) The four original beams were split into two identical sets, wherein one set was bounced off a simple mirror which changes the OAM values from positive (i.e., clockwise) to negative (i.e., counterclockwise) and vice versa, resulting in 8 orthogonal OAM beams (4 positive and four negative). (ii) The 8 OAM beams were split into two identical sets, with each set placed on one of two orthogonal polarization states and resulting in 16 beams. (iii) The 16 beams were split into two identical sets again, and each set was transmitted with a different radius using telescopic optics, two groups of 16 OAM beams were transmitted on two distinguishable (though not strictly orthogonal) groups of concentric rings. These 32 data streams each carried 80 Gbit/s, allowing for a total capacity of 2.56 Tbit/s and a spectral efficiency of 95.7 bits per sec per Hz.

The multiplexing of multiple orthogonal data-carrying OAM beams can be likened to the ability to multiplex and copropagate many different data-carrying beams that each resides on a different wavelength, known as wavelength-division multiplex-

ing (WDM) (9). WDM enabled dramatic advances in communication systems capacity over the past >20 years, such that more than 100 different wavelength channels could each simultaneously carry an independent data stream in a system. Importantly, OAM multiplexing is compatible with wavelength multiplexing and represents another orthogonal degree-of-freedom to multiply data-carrying capacity. Indeed, each independent wavelength can be encoded with multiple independent OAM values (10).

One of the key challenges in free-space OAM transmission is turbulence in the atmosphere (11, 12). For situations that require high capacity or spectral efficiency over relatively short distances, this approach could be appealing. On the other hand, there are also exciting opportunities for long-distance satellite-to-satellite communications in space, where turbulence is not an issue.

There are two crucial issues regarding the potential for using OAM in future communication systems. First, most key OAM experiments have occurred in free space, and yet much of the current high-capacity communication occurs in optical fiber. Therefore, there is appreciable interest in achieving high capacity using OAM in fiber. Although conventional fiber does not readily support a twisting OAM beam, there are reports of ring-like fiber that can support OAM propagation (13). Second, future systems would ben-

efit greatly by having compact, efficient, and cost-effective methods of generating, multiplexing, and recovering beams with different OAM values, and work is progressing on integrated techniques (14, 15).

Will OAM be used in future communication systems? Although the field is young and challenges remain, the prospects are indeed exciting.

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CLIMATE CHANGE

Ice Sheets in Transition

Peter U. Clark

It is now well known that periodic variations in ice-age climate correspond to variations in Earth's orbit around the Sun (1, 2). However, some notable aspects of this climate variability appear to have little or no relation to orbital forcing. One such example is the middle Pleistocene transition (MPT), which began at some time after 1.25 million years ago. Before the MPT, climate was dominated by low-amplitude 41,000-year cycles; over the course of the MPT, these were replaced by dominant high-amplitude 100,000-year cycles in the absence of any change in orbital forcing (see the first figure) (3). On page 704 of this

issue, Elderfield *et al.* (4) provide important new insights into ice-age variability and the origin and timing of the MPT.

Any assessment of the cause of climate variability, as reconstructed from the geological record, is only as good as our understanding of the climate signal inferred from that record. Interpretation of a record commonly used to characterize ice-age variability—the ratio of ^{18}O to ^{16}O preserved in deep-sea carbonate microorganisms, referenced to a standard and reported as $\delta^{18}\text{O}_\text{C}$ —is no exception.

The $\delta^{18}\text{O}_\text{C}$ proxy is often inferred to largely reflect changes in global ice volume, which change the $\delta^{18}\text{O}$ of seawater ($\delta^{18}\text{O}_\text{w}$); however, it also reflects temperature. Distinguishing between these two controls is critical for understanding two key elements of

Temperature reconstructions from the deep sea reveal how the global ice volume has varied over the past 1.5 million years.

Earth's climate system: ice sheets and ocean heat storage. Yet, making that distinction has proven difficult because the $\delta^{18}\text{O}_\text{C}$ signal from ice sheets and temperature likely varied over time and space and because deep-water masses have differing $\delta^{18}\text{O}$ signals. Understanding of the deep-sea $\delta^{18}\text{O}_\text{C}$ signal has thus remained incomplete.

Elderfield *et al.* now show how the temperature component can be deconvolved from a $\delta^{18}\text{O}_\text{C}$ record spanning the past 1.5 million years. To do so, the authors first applied an independent proxy of temperature to a deep-sea foraminiferal record from the southwest Pacific Ocean near New Zealand [Ocean Drilling Program (ODP) site 1123]. The proxy, Mg/Ca, is based on observations showing that during marine carbonate precipitation, Mg increases with

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Ice-sheet transitions. (A) The average $\delta^{18}\text{O}_c$ record based on 57 $\delta^{18}\text{O}_c$ records from the world's oceans (10). The earlier part of the record is characterized by dominant low-amplitude 41,000-year cycles, whereas the latter part is dominated by high-amplitude 100,000-year cycles. The transition between the two intervals occurs during the middle Pleistocene between ~1.2 million years ago and 0.7 million years ago. It is thought to be associated with increasing maxima in peak ice volume. (B) Distribution of power through time associated with the changes in the Earth's orbital parameters of precession (P, 19,000 and 23,000 years), tilt (T, 41,000 years), and eccentricity (E, 100,000 years) (11). Red and purple colors indicate a greater concentration of power for a given frequency, green colors indicate reduced power, and blue indicates little or no power. Note the absence of change in orbital forcing during the MPT, as well as the absence of significant power in the eccentricity band during the time of maximum signal at this frequency (100,000 years) in the $\delta^{18}\text{O}_c$ average. (C) The $\delta^{18}\text{O}_w$ record from ODP site 1123 in the southwest Pacific, reported by Elderfield *et al.* In contrast to the middle Pleistocene transition from the $\delta^{18}\text{O}_c$ average, the transition here occurs more abruptly at ~0.9 million years ago.

temperature (5). Measuring Mg/Ca on the same microfossil samples as $\delta^{18}\text{O}_c$ allows, by simple subtraction, the direct derivation of the $\delta^{18}\text{O}$ of the seawater in which the foraminifera lived.

The Mg/Ca proxy is now widely used for reconstructing sea-surface temperatures and salinities (6), but two issues have limited its application to the deep sea (7–9). First, the calibration for the low temperatures of the deep sea may have larger uncertainties than for the surface ocean. Second, the ocean's carbonate ion concentration may modulate the uptake of metals during carbonate precipitation. Elderfield *et al.* circumvent these issues by using a species (*Uvigerina* spp.) that has a reasonably well-constrained temperature calibration and is largely isolated from the carbonate ion effect.

Their record clearly shows the characteristic features of ice-age variability, including the change from 41,000-year cycles to

100,000-year cycles during the MPT. Understanding the cause of the MPT is of particular interest not only because the 100,000-year world emerged in the absence of any change in orbital forcing, but also because the orbital forcing at the 100,000-year frequency (eccentricity) is the weakest orbital parameter (see the first figure, panel B) (2).

But exactly when did the MPT occur, and how quickly? Individual $\delta^{18}\text{O}_c$ records disagree widely on this, in part because each record may be influenced by local variability in water mass hydrography. Averaging of records improves the signal-to-noise ratio. The most comprehensive averaging of $\delta^{18}\text{O}_c$ records to date (10) suggests that the MPT was gradual, beginning ~1.2 million years

ago and ending by ~0.7 million years ago (11) (see the first figure, panel A). Comparison of Elderfield *et al.*'s $\delta^{18}\text{O}_c$ record from site 1123 to the global $\delta^{18}\text{O}_c$ average shows, however, that their representations of the MPT differ. Given that the temperature record from site 1123 does not show any unusual change across the MPT, this difference must reflect a change in the $\delta^{18}\text{O}_w$ at the site (see the first figure, panel C).

Elderfield *et al.* summarize several lines of evidence suggesting that the $\delta^{18}\text{O}_w$ signal is not affected by changes in local hydrography and thus represents global sea level. In contrast, the relative controls of temperature and $\delta^{18}\text{O}_w$ on the $\delta^{18}\text{O}_c$ average are unconstrained. Furthermore, the authors suggest that the $\delta^{18}\text{O}_c$ average may be biased by a North Atlantic signal and thus not be representative of much of the world's oceans.

Whether Elderfield *et al.*'s $\delta^{18}\text{O}_w$ record is representative of the world's oceans remains to be seen. But their highly resolved data constitutes considerable progress in characterizing sea-level and deep-ocean temperature variability during the ice ages, with several particularly notable insights. The record supports the long-standing notion that global ice sheets during the maxima of the 41,000-year cycles were smaller than during the maxima of the 100,000-year cycles. It also shows that the deep sea cooled to nearly freezing temperatures early in any given gla-

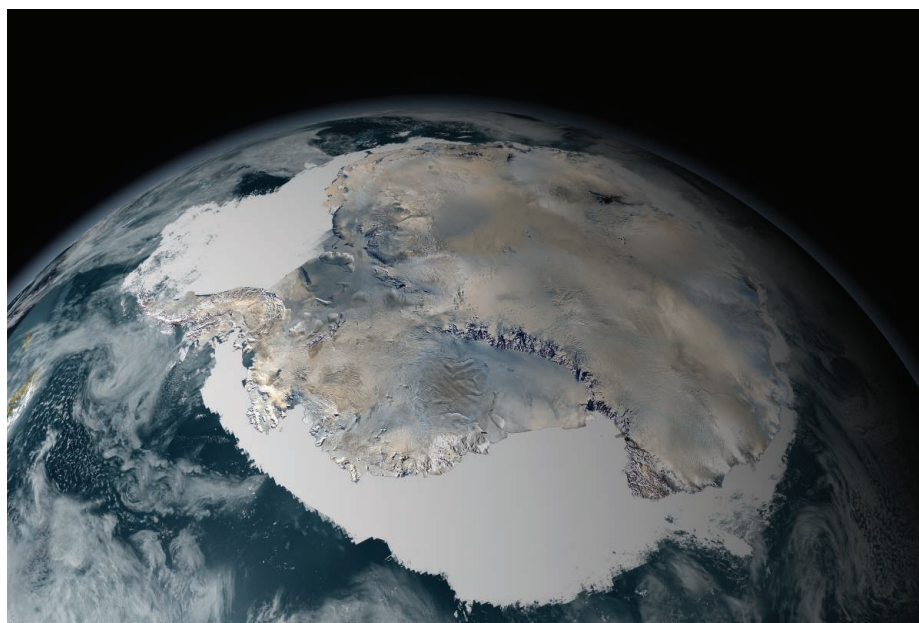
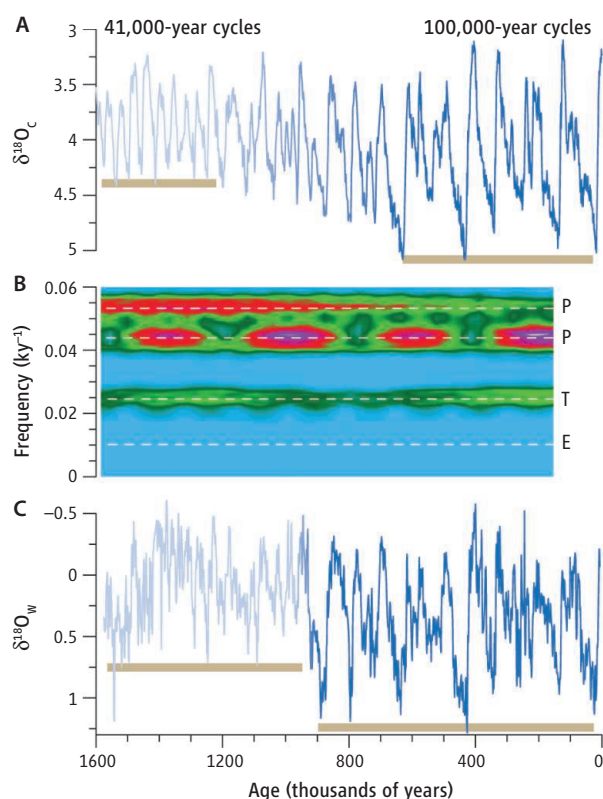


Image of Antarctica. According to the new study by Elderfield *et al.* (4), expansion of the Antarctic Ice Sheet was responsible for the middle Pleistocene transition.

cial cycle, whereas global ice volume typically increased gradually (12).

With respect to the origin of the MPT, Elderfield *et al.* conclude that the increase in global ice volume during this time occurred largely through expansion of the Antarctic Ice Sheet (see the second figure), possibly due to Southern Hemisphere seasonal insolation forcing. This conclusion stands in marked contrast to previous hypotheses for the MPT (11). Confirmation of these hypotheses will require generation of sim-

ilar-quality deconvolved $\delta^{18}\text{O}_\text{C}$ data sets, which should help to better understand the range of regional variability in deep-ocean temperature and $\delta^{18}\text{O}_\text{W}$.

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MEDICINE

Leveraging Shear Stress to Bust Clots with Nanoparticles

Erin Lavik and Jeffrey Ustin

In 1987, the first clinical trial of recombinant tissue plasminogen activator (tPA) for the treatment of acute stroke commenced and led to U.S. Food and Drug Administration approval in 1996 (1). tPA is used as soon after the stroke as possible, within the first 4 hours, to dissolve clots and restore blood flow. It has revolutionized treatment of strokes, heart attacks, and pulmonary embolisms; however, it is not without risk. On page 738 in this issue, Korin *et al.* (2) describe a method they have developed for targeting tPA to clots to reduce the side effects while restoring blood flow.

tPA is a naturally occurring enzyme that binds with fibrin, the mesh of a clot, and converts plasminogen to plasmin (3), leading to clot breakdown. Administration of tPA has become a standard of care in the field for breaking down clots in strokes,

heart attacks, and embolisms. However, it increases the risk of bleeding in the brain as well as in other tissues, which can be deadly (4).

Ideally, one would like to be able to administer the drug locally to the clots and avoid having the drug circulate to other tissues, increasing the risk of bleeding events. Several groups have tried to encapsulate tPA and target the drug to clots using peptides (5), electrostatic interactions (6), or ultrasound-triggered release (7). However, it has remained challenging to get meaningful amounts of tPA delivered efficiently to the clots.

Korin *et al.* describe an alternative approach to clot busting with tPA. Rather than relying on molecular targeting or application of ultrasound, they leverage the difference in shear forces between normal vessels and blocked vessels to trigger delivery of tPA. The differences in shear forces cause aggregates of nanoparticles coated with tPA

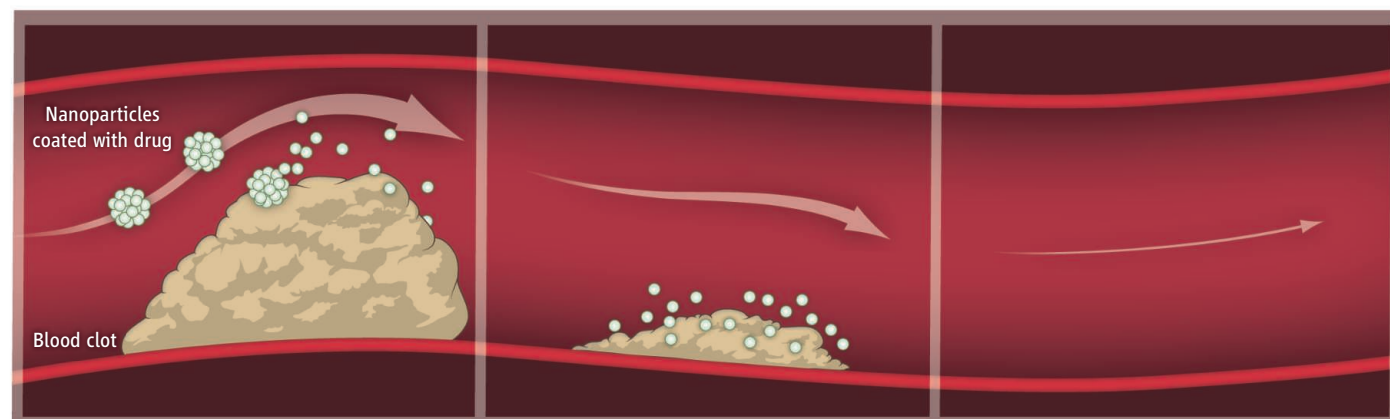
Nanoparticle aggregates break apart to release drugs under increased shear stress in blocked blood vessels for improved treatment of stroke, heart attacks, and embolisms.

to burst apart (see the figure), which then breaks down the clots.

The nanoparticles are based on poly(lactic-co-glycolic acid) (PLGA), the material used in degradable sutures. A spray-drying technique was used to assemble the particles into aggregates, which were then chemically modified with a poly(ethylene glycol) (PEG) spacer to which biotin is coupled. tPA is also modified with biotin, and biotin-avidin binding is used to attach the tPA to the surface of the particles.

Shear stress between 10 and 100 dynes/cm² causes the aggregates to break apart into nanoparticles. One of the key find-

Targeting drugs by shear stress. A clot in the blood vessel leads to an increase in local shear stress (indicated by the arrow). This triggers the targeted disassembly of nanoparticle aggregates (circles) coated in tissue plasminogen activator (tPA) into individual nanoparticles, which then dissolve the clot and restore blood flow.



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ings of Korin *et al.* is that administration of the aggregates cleared clots induced by ferric chloride in mouse arteries. Administration of tPA at the same dose (50 ng), aggregates without tPA, predissociated tPA-coated aggregates, or fused aggregates that are coated with tPA did not have appreciable effects. Notably, neither dispersed nanoparticles nor fused aggregates disrupted the clots. The authors observe that the aggregates bind to the clots and begin dissolution, leading to further increases in shear stress and deployment of the nanoparticles to complete clot dissolution.

Korin *et al.* investigated the tPA-coated aggregates in several clinically relevant models of vessel blockages, including a number of variations on pulmonary emboli and dosing immediately after clot formation and 30 min later. Large clots in the lungs are lethal, and administration of the tPA-coated aggregates improved survival.

tPA is clearly an effective therapy. The big question is whether the aggregates reduce complications. The aggregates are relatively large (2 to 5 μm) and are cleared within minutes of administration. Such large particles are often considered risky for intravenous administration because they can cause clots. The ability of these aggregates to break apart limits that risk substantially. Still, the concerns with tPA must be considered, and

the authors note that they are using tPA on the particles at 1/100th of the concentration used in the clinic, and no adverse bleeding events have been seen thus far.

The work by Korin *et al.* is certainly exciting, but is still in the early stages. It is challenging to make very tightly controlled aggregates of nanoparticles at scale, and that will be a critical part of moving this technology forward. Streptavidin-biotin chemistry is very versatile, but as the authors note, it is associated with immune responses that can limit translation.

Approximately 800,000 people will experience a stroke this year in the United States (8), and worldwide, 5.5 million people will die from stroke-related causes (9). Pulmonary embolisms and deep vein thrombosis are estimated to be on par with the number of strokes (10), and heart attacks are the leading cause of death worldwide (11). Thus, there is a need for technologies that are safe, simple and can be easily administered to break up clots. The use of shear forces to trigger these aggregates is relatively simple and very effective, at least in these models, and is a potentially extremely powerful technique for targeting blocked vessels.

More broadly, the study by Korin *et al.* shows that we need to think outside the box in targeting nanotechnologies in the body. There has been much focus in the field on

finding the right, high-affinity molecule or molecules to target nanoparticles. Leveraging other phenomena such as differences in shear stresses in the targeting regions may lead to more successful enrichment of particles at the target site with fewer systemic complications (12). The authors provide compelling early data for developing new treatments for clots, but their work also marks a change in approach that broadens the potential of nanomedicine.

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CHEMISTRY

Low-Temperature Oxidation of Methane

Robert J. Farrauto

Since automobile emissions standards were written into the U.S. Clean Air Act of 1970, methane has been absent from the list of pollutants covered by such regulations (1–4) because it is unreactive in photochemical smog-generating reactions. Today we understand that methane is a potent greenhouse gas. Future transportation emission standards are therefore expected to include methane. On page 713 of this issue, Cargnello *et al.* (5) report a new approach in catalyst structure that may help to address methane emissions in the automobile exhaust within the temperature range

required for emission control. Low-temperature methane combustion is also important for catalyst-assisted combustion in gas turbines fueled with natural gas.

The removal of methane from transport emissions is a major challenge for automakers and catalyst suppliers. In the past four decades, there has been little progress in developing catalysts that can initiate methane oxidation at the required temperatures. Palladium oxide (PdO) supported on various materials has been shown to initiate oxidation of methane at about 400°C, but the expected requirement will be <300°C in the exhaust of a lean-burn engine. Furthermore, exhaust temperatures of 800° to 850°C during fast driving lead to decomposition of PdO to less active Pd, as well as sintering. The latter is

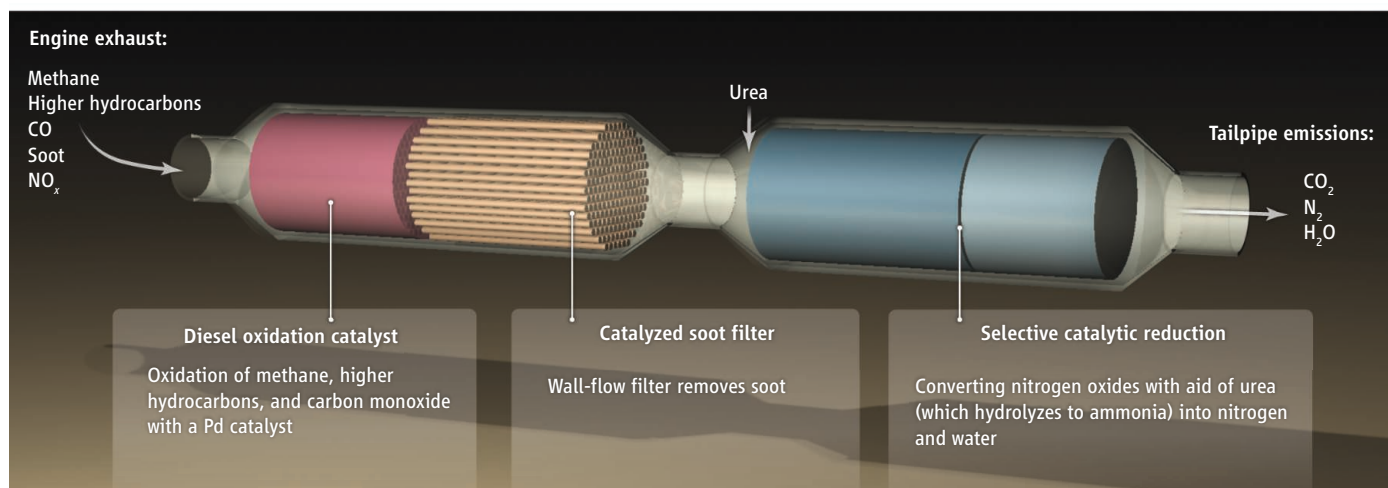
Catalysts prepared by modular assembly of subunits into a functionalized support show exceptional activity and stability for methane oxidation.

irreversible even when Pd reverts to more active PdO at lower temperatures.

Cargnello *et al.* now report a catalyst that has low-temperature activity and stability under thermal conditions that simulate an automobile exhaust system. The PdO is encapsulated in a cerium oxide (CeO₂) matrix, resulting in enhanced performance relative to the traditional supported PdO catalysts. This raises the potential for a catalytic solution for removing a powerful greenhouse gas from lean-burn engine exhausts. This result alone will stimulate new thinking in catalyst development and system design.

However, the exhaust contains harmful combustion products, such as sulfur oxides and oil-additive elements such as compounds of phosphorus, zinc, and calcium,

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Requirements for the diesel oxidation catalyst. Catalyzed monoliths in the exhaust of a future diesel engine are likely to comprise a diesel oxidation catalyst, a catalyzed wall flow filter, and a NO_x reduction system. The diesel oxidation catalyst must oxidize methane, carbon monoxide, higher hydrocarbons, and

nitric oxide. Cargnello *et al.* report a PdO catalyst that shows high activity and stability, under thermal conditions that simulate an automobile exhaust system but whether it can be successfully integrated into a catalyzed monolith while avoiding sintering and poisoning remains unknown.

all of which poison most catalysts, especially PdO and CeO₂. The presence of 5% steam in lean-burn engines can also inhibit catalytic reactions and cause excessive sintering of the catalytic components and their carriers. Furthermore, catalysts must be prepared cost-effectively in high volumes as a washcoat on a monolith (6). It remains to be shown whether Cargnello *et al.*'s catalyst can survive under these conditions.

The severity of the duty cycle for a typical exhaust catalyst, together with an expected lifetime of ~400,000 miles for diesel trucks in the United States, is challenging for any catalyst. Clearly this can only be achieved with enhanced catalysts, an advanced engine control strategy, and improved fuel quality. In the diesel engine, the oxidation catalyst must achieve simultaneous complete oxidation of not just methane but also carbon monoxide (CO), higher hydrocarbons, and nitric oxide (NO) to nitrogen dioxide (NO₂). In practice, the diesel oxidation catalyst will likely be placed in a position near the engine (see the figure) to elevate the inlet temperature to that required for methane oxidation.

As difficult as these criteria may sound, the combination of improved catalysts, engine control strategies, and improved fuel quality has been successful for the gasoline three-way catalytic converter, which has been in use in cars since 1981 to reduce NO_x emissions and to oxidize CO to CO₂ and higher hydrocarbons to CO₂ and H₂O (4). The current U.S. standards require this catalyst to meet stringent emission regulations for 150,000 miles of consumer driving; matching this requirement is a major technological accomplishment.

Natural gas-fired turbines for power generation would also benefit from a catalyst that can oxidize methane at low temperature. In the early 1970s, Engelhard Corporation (bought by BASF Corporation in 2006) patented catalyst and process technology with the promise of low CO, hydrocarbon, and NO emissions without post-emission treatment (7). This technology requires the catalytic oxidation of a mixture of natural gas (mostly methane) and air below the flammability limits. At a gas temperature of ~650° to 700°C, the mixture becomes flammable, and complete homogeneous combustion occurs at gas temperatures approaching 1300°C. The hot combustion gas is then directed to a turbine for power generation. The technology would make mechanical burners obsolete and eliminate the need for selective catalytic reduction of NO_x. However, no catalyst currently exists that can convert methane at the required temperature, and this technology has never been commercialized.

A major requirement for the catalyst is that it must light off methane at the air compressor discharge temperature of 340°C at 13 atmospheres. The best candidate is PdO on various carriers, but as discussed above, it can become deactivated through conversion to Pd and sintering. The temperature for decomposition of PdO to Pd (~800°C at atmospheric pressure) is much higher than that at which it is reformed to PdO (550°C) (8, 9). This hysteresis creates a window of little activity. Cargnello *et al.*'s catalyst shows very little hysteresis, meaning that the catalyst reforms active PdO at higher temperatures than previous catalysts. This

observation suggests that in their catalyst, O₂ is activated by the CeO₂ during the cool-down; the dissociated O atoms then react to form active PdO.

After 40 years of catalyst failures, there is reason to be hopeful. The low-temperature methane catalyst reported by Cargnello *et al.* is an important step toward two main applications: abatement of methane from internal combustion engines for emission control, and catalytically assisted homogeneous combustion. However, demonstration in real applications will require additional catalytic and system breakthroughs in sintering and poison resistance required for long-term performance. I hope others will take up the challenge to begin addressing these important issues.

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RETROSPECTIVE

Elinor Ostrom (1933–2012)

Rick K. Wilson

Elinor (Lin) Ostrom, Distinguished Professor at Indiana University, died on 12 June 2012 at the age of 78. Her husband and intellectual partner, Vincent Ostrom, died on 29 June 2012 at the age of 92. Lin was a Fellow of the American Association for the Advancement of Science and a member of the United States National Academy of Sciences. In 2009, Lin was awarded (with Oliver Williamson) the Nobel Memorial Prize in Economic Sciences, the first (and only) woman to win the prize. Doubly interesting is that she was not an economist, but a political scientist. While proud of her discipline, at heart, she considered herself a social scientist.

Lin was from Los Angeles, California, and received her undergraduate, masters, and doctoral degrees from the University of California, Los Angeles. Her early career began with a study of water resources in Southern California, where she addressed the puzzle of why some water districts were able to self-organize to stanch the depletion of an aquifer, while others failed. The answer was in the institutional detail of how groups organized. Soon thereafter, Lin and her husband Vincent sought academic positions. As she reminded me before my tenure, it was important to have a back-up plan. Theirs was to open a wood-working shop in Haight-Ashbury in 1965. What cabinet making lost, Indiana University gained by offering them positions, although Lin's was initially an adjunct position.

In 1973, Lin and Vincent founded the Workshop in Political Theory and Policy Analysis, which welcomed students and faculty alike from all social science disciplines, as well as the occasional mathematician and anyone with an interest in theoretical reasoning about policy problems. Two words characterized the Workshop: collaboration and co-production. Students were included on projects on equal footing with faculty. If you thought you had a contribution to make, you were listened to and were expected to defend your position. Lin's boisterous laugh and the sparkle in her eye made it easier to accept the fact that your new model was going nowhere. When you made a contribution, she welcomed it with open arms and quickly pressed you to do more.

Out of the Workshop came an amazing array of projects with Lin at the center. In the 1970s, she questioned whether centralized, consolidated governments could better provide basic citizens services than a decentralized, self-organized system she later came to call "polycentric." To test her ideas, she undertook a major study of police services across different jurisdictions. In her usual fashion, Lin assembled a large group of students, built comprehensive measures, made friends with everyone, and rode into the field with on-duty police officers. The data supported her insight: Smaller police departments in more fragmented jurisdictions provided better services than large consolidated departments.

In the 1980s and 1990s, Lin returned to the problem of the "tragedy of the commons." This is a well-known dilemma in which no one can be prevented from appropriating a resource (e.g., fishing), but when appropriated, the resource disappears. This gives everyone an incentive to take as much as possible, and it leads to the resource crashing (e.g., overfishing). The usual solution appeals to a central authority to solve the dilemma. Yet the track record for centralized solutions has been abysmal. Lin's work pointed out that local users are in the best position to solve such a dilemma. This is accomplished by adopting social, political, and economic institutions that match the local culture, embody local norms, and fit the resource. She amassed thousands of case studies and explored systematic regularities by developing formal models and testing them with laboratory experiments. That work turned the common wisdom on its head.

In the 2000s, Lin traveled farther afield, working with different groups on a wide variety of dilemmas, including farmers in Nepal and foresters in Nigeria and Kenya. The Nobel Prize gave her a marvelous soapbox on which to stand. She admitted that, while she once spent time convincing low-level

The only woman to win the Nobel Prize in economics studied the problem of the "tragedy of the commons" for the betterment of mankind.



bureaucrats, now she spoke to heads of agencies, and they listened. Even after she became ill with pancreatic cancer, Lin continued a rigorous travel schedule. Weeks before she died, she was the keynote speaker for the International Association for the Study of the Commons held in Mexico City. Everywhere her message was the same: There is no universal panacea; encourage the development of local institutions. Although the tragedy of the commons can be solved, there is no set formula, and the right solution means

choosing carefully from a rich array of institutional solutions.

Her colleagues and friends wondered how she managed to accomplish so much. There were three answers. Lin was awake and working by 3 am. She and Vincent escaped every summer to their remote cabin on Manitoulin Island on Lake Huron, devoting their time to writing. And Lin read voraciously, far beyond the confines of her discipline. When she tackled a problem, she enlisted a team of students and staff to assemble a huge bibliography, which she then read and synthesized. Emblematic of the scope of her scholarship was her 1998 Presidential address to the American Political Science Association, in which she urged the community to move beyond standard boundaries and embrace important findings from biology and neuroscience.

Lin's legacy will continue through the workshop and through her many students and collaborators. Her students will remember the lessons that she taught us about doing science. Visitors to the workshop will remember the excitement of the intellectual exchange. Co-authors will remember her inspiration and exacting standards. Those fortunate enough to have met her will remember a kind, generous person who sought answers to many questions with the goal to improve policy choices. She will be sorely missed. 10.1126/science.1227725



WORKING WITH WASTE

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INTRODUCTION

More Treasure Than Trash

WASTE IS FAR FROM A GLAMOROUS SUBJECT, BUT IT CAN'T BE AVOIDED. DEPENDING on lifestyle and consumption patterns, each of us can generate tons of waste over our lifetimes, from longstanding sources such as table scraps, old newspapers, and bottles and cans to the ever-growing stream of consumer electronics that nowadays approach obsolescence mere months after purchase. The total really skyrockets if you include the farm, mine, and industrial wastes generated to produce food, power, and products in the first place.

As this special issue of *Science* highlights, however, trash is often treasure—a feedstock that cannot be overlooked as an expanding world population tries to use resources more efficiently and reduce the strain that our consumption places on natural systems. Those heaps of crop leftovers and yard clippings, for instance, can offset petroleum as a source of the commodity chemicals used to make fuels, medicines, and cosmetics, if systems are in place to collect and distribute them. Plastics and metals can be recycled multiple times. One of our newest waste concerns, the carbon dioxide gas produced by burning fossil fuels, could have value if captured and used creatively. Even the wastewater we generate can be transformed into a source of energy and clean water, and water treatment cost-effectiveness is on the rise.

Of course, these aren't all novel concepts; people have been imaginatively working with waste for millennia, from using human waste to create "black soil" in farm fields to melting down broken swords to make plowshares. But as the News stories, Reviews, and Perspectives in this issue demonstrate, working with waste is an increasingly complex challenge. To minimize the amount of waste we generate and wring the most value out of the trash we create requires a mix of smart science, practical policy, and appropriate technology. It's not enough to understand chemistry and materials science, for instance, because psychology, politics, and economics also play a big role in how we come to terms with our waste.

Still, the snapshots offered in this special section suggest that we are making some progress, even as sobering challenges remain. A series of infographics helps put these developments in a broader context but also reminds us that, in much of the world, collecting reliable waste statistics remains a work in progress. Online content, including a podcast and several videos, adds additional perspective.

Together, the reporting suggests that, ultimately, we can make our waste more treasure than trash. But it also reveals that to achieve that goal, we'll have to work with our waste more than ever. Luckily, a growing number of researchers appear to be interested in getting their hands dirty.

— NICK WIGGINTON, JAKE YESTON, DAVID MALAKOFF

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www.sciencemag.org/special/waste

Wireless. Shredded plastic from the insulation of discarded electrical cables at a processing facility in Montpellier, France.

Science

WORLD OF WASTE

One person's trash, the saying goes, is another person's treasure. But when it comes to waste statistics, there's a big difference: While governments tend to tally treasure down to the last penny, they aren't so meticulous about accounting for rubbish. As a result, the scope and reliability of waste statistics vary widely around the world. In general, poorer, developing nations have the softest numbers. But even wealthy, developed countries can have big data

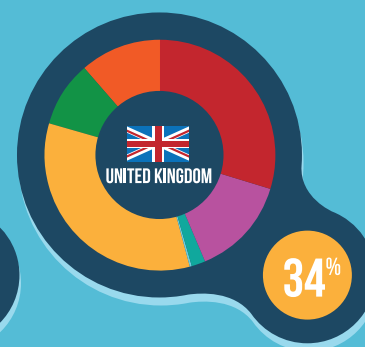
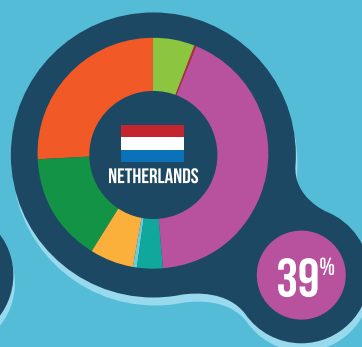
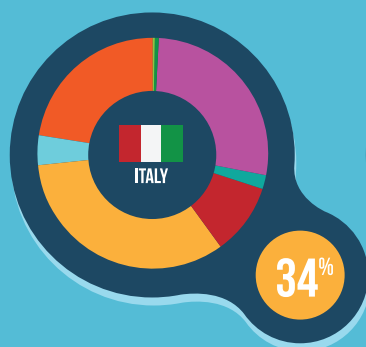
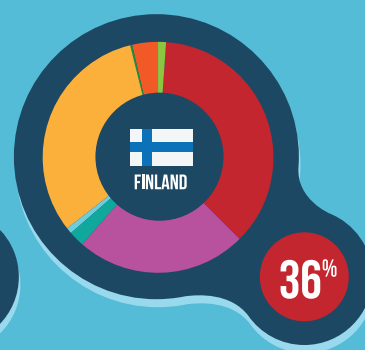
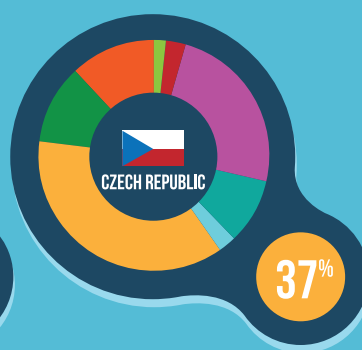
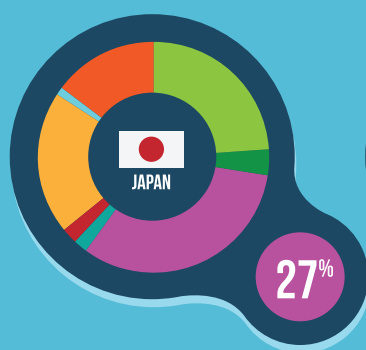
gaps. In the United States, for example, battles over waste policy have complicated government efforts to document some waste streams, and budget cuts threaten programs that try to calculate how materials flow through the economy. Further complicating matters, nations sometimes use different waste definitions, making meaningful comparisons difficult. Still, enough data exist to draw some insights into where the world's waste is coming from, where it is going, and how waste streams are changing. And the numbers suggest that local economic, social, and geographic factors can play a big role in waste management, leading nations to often take very different approaches.

THE COMPOSITION OF WASTE STREAMS CAN VARY WIDELY BY COUNTRY

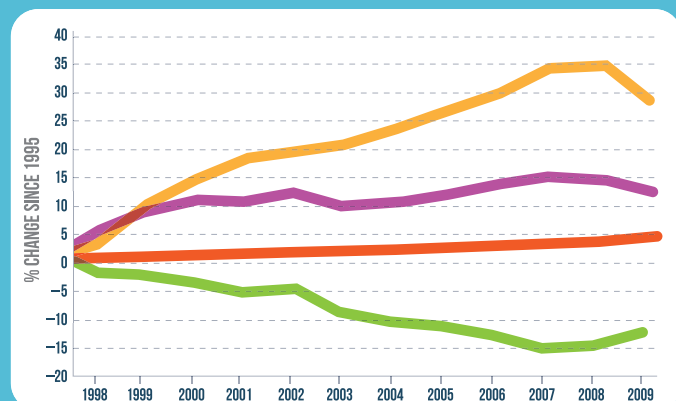
When you think of waste, piles of manure next to a farm or heaps of sludge from a water-treatment plant may not immediately come to mind. In some nations, however, agriculture or water treatment can generate a sizable proportion of the overall estimated waste stream (*right*). In others, the biggest waste producers might be construction, mining, or municipal waste—the hodgepodge of household and business waste produced by people living in cities and towns.



SOURCE: OECD/OCDE



WASTE & WEALTH: THERE APPEARS TO BE A LINK



The richer you get, the more you can afford to waste. That's the general picture that emerges from studies of the links between population, wealth, and trash. Although a rising population can drive an increase in the amount of trash, the bigger factors appear to be wealth and consumption, with people in richer nations producing, on average, more waste per capita than those in poorer ones (*below*). But aggressive waste-minimization efforts in some regions, such as the European Union, are attempting to "decouple" waste from wealth, leading to lower waste generation per unit of GDP (*above*).

- GROSS DOMESTIC PRODUCT
- MUNICIPAL WASTE GENERATED
- POPULATION
- MUNICIPAL WASTE GENERATED PER EURO (GDP)

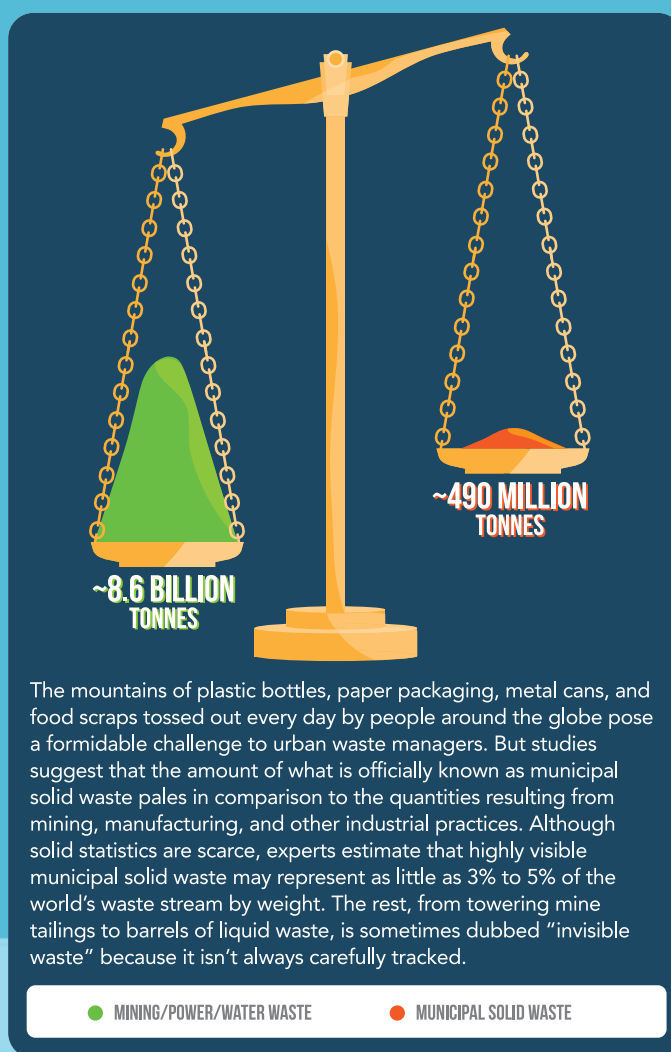
SOURCE: EUROSTAT

WASTE GENERATION VARIES BY INCOME LEVEL

NATIONAL INCOME LEVEL	WASTE GENERATION PER CAPITA (KG/CAPITA/DAY)		
	LOWER BOUNDARY	UPPER BOUNDARY	AVERAGE
HIGH	0.70	14	2.1
UPPER MIDDLE	0.11	5.5	1.2
LOWER MIDDLE	0.16	5.3	0.79
LOWER	0.09	4.3	0.60

SOURCE: WHAT A WASTE: A GLOBAL REVIEW OF SOLID WASTE MANAGEMENT (2012)/THE WORLD BANK

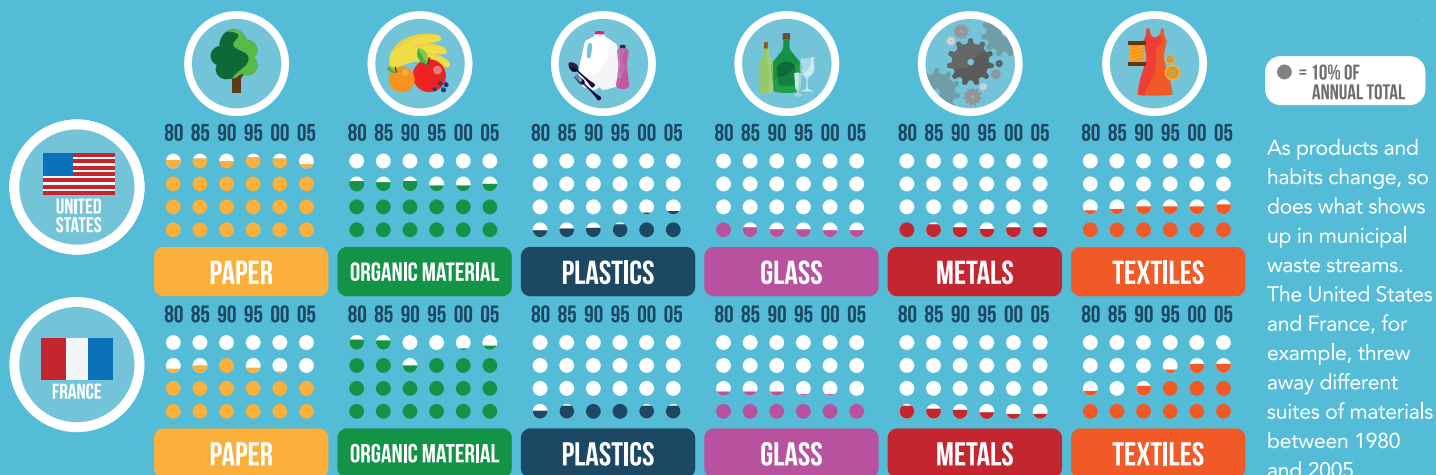
WHAT MANY PEOPLE THINK OF AS TRASH IS A SMALL PART OF GLOBAL WASTE



The mountains of plastic bottles, paper packaging, metal cans, and food scraps tossed out every day by people around the globe pose a formidable challenge to urban waste managers. But studies suggest that the amount of what is officially known as municipal solid waste pales in comparison to the quantities resulting from mining, manufacturing, and other industrial practices. Although solid statistics are scarce, experts estimate that highly visible municipal solid waste may represent as little as 3% to 5% of the world's waste stream by weight. The rest, from towering mine tailings to barrels of liquid waste, is sometimes dubbed "invisible waste" because it isn't always carefully tracked.

SOURCE: CYCLOPE

DIFFERENT SOCIETIES, DIFFERENT MUNICIPAL SOLID WASTE MATERIALS



THE FATE OF MUNICIPAL SOLID WASTE: BURY, BURN, OR RECYCLE?

How nations deal with their municipal solid waste can come down to geography, economics, and politics. Live in a nation with lots of cheap, open land near big cities? Burying your municipal solid waste in a landfill might be the cheapest option. But if real estate is expensive, burning it in an incinerator—and possibly generating some electricity with the heat—might be the choice. Or if there's political support and a reliable market, the most valuable waste materials could end up being recycled. Such factors help explain why the fate of waste varies greatly among nations, with some burying a majority of their documented municipal solid waste in landfills (*top, right*), while others tend to burn (*middle*) or recycle it (*bottom*).

54.3%



1.7%

74%



0.5%

49.2%

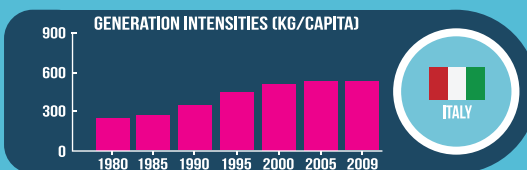
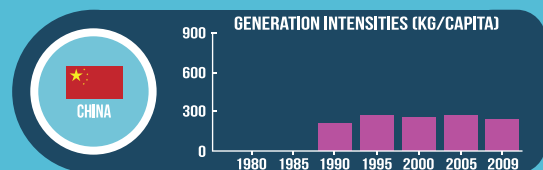
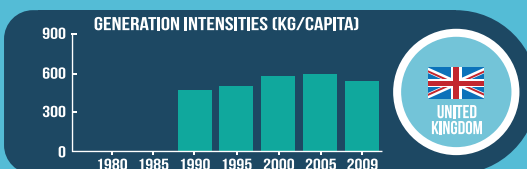
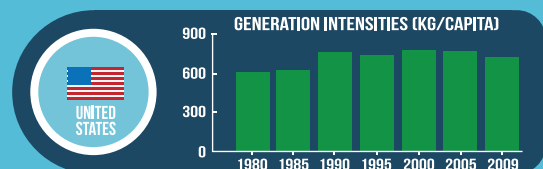
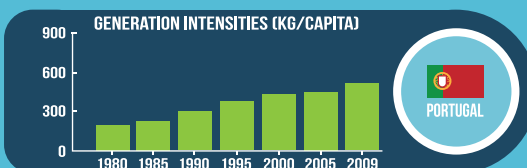
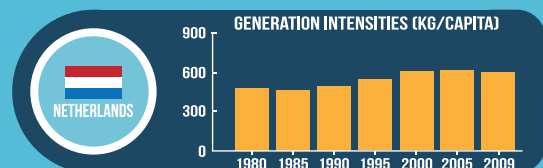
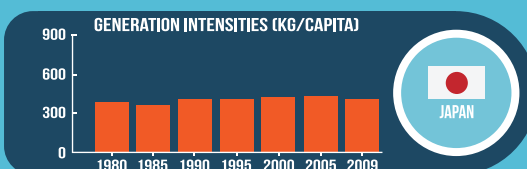
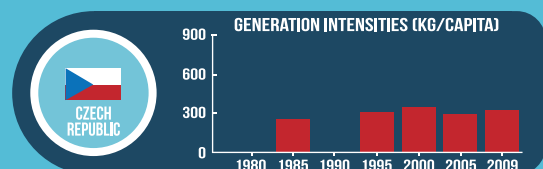


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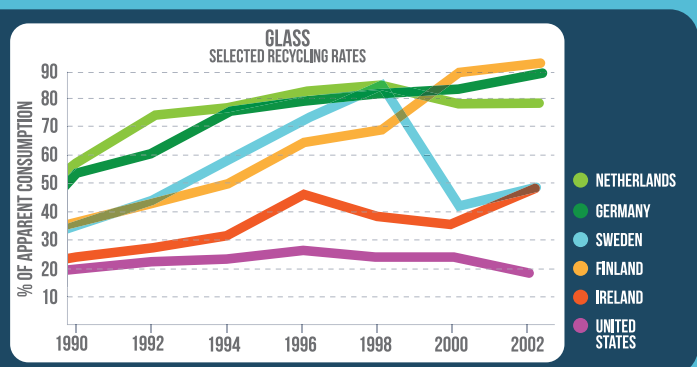
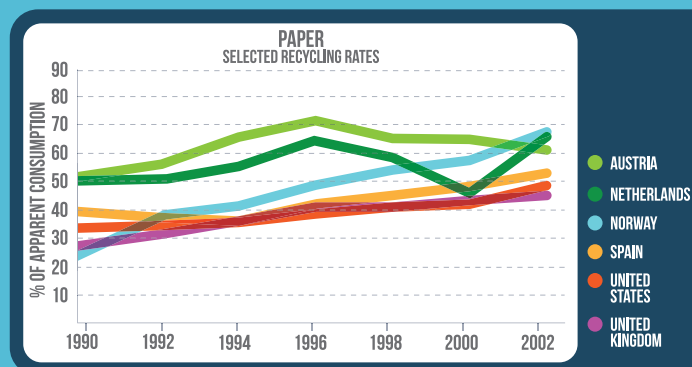
SOURCE: ALL DATA FROM OECD/OCDE



AS TIME PASSES, THE PRODUCTION & FATE OF MUNICIPAL SOLID WASTE IS CHANGING



As waste management becomes a more pressing global issue, some nations are trying to reduce their "waste intensity"—the average amount of municipal solid waste produced per person—with mixed results (*top graphs*). Governments are also moving to adopt policies that create incentives to recycle more material, such as paper and glass (*below*). Although global economic trends can sometimes weaken markets for recycled materials, many nations are reusing more of their most valuable waste products than they did decades ago.



SOURCE: ALL DATA FROM OECD/OCDE





GARBOLGY 101: GETTING A GRIP ON WASTE

Most people choose to ignore it, but managing waste is a pressing and contentious issue

PEOPLE WHO WORK WITH WASTE FOR A living seem to have a special passion for their subject. And they aren't fazed by its complexity. Those traits help explain why Bill Davidson found himself tapping away at his home computer one Sunday morning more than a decade ago.

A consultant "got lost" trying to write a report on waste management in Montgomery County, Maryland, recalls Davidson, now section chief for strategic planning in the county's Division of Solid Waste Services. So the mechanical engineer, who once crunched numbers for the congressional Office of Technology Assessment, decided to try his hand at depicting what happens to all the waste generated each year by those who live, work, and play in these affluent suburbs of nearly 1 million people outside Washington, D.C.

His solution was an elegant flow chart that tracks 15 streams of the county's detritus, which last year totaled 1.34 million tons (see diagram, p. 669). It depicts the ultimate fate of every chicken bone, diaper, cereal box, beer can, plastic bag and bottle, broken toy, mattress, and grass clipping discarded by this racially mixed, highly educated, and relatively environmentally aware suburban community.

Davidson and his colleagues were able to devise such a flow chart because their employer takes waste management seriously. Recycling is mandatory in Montgomery

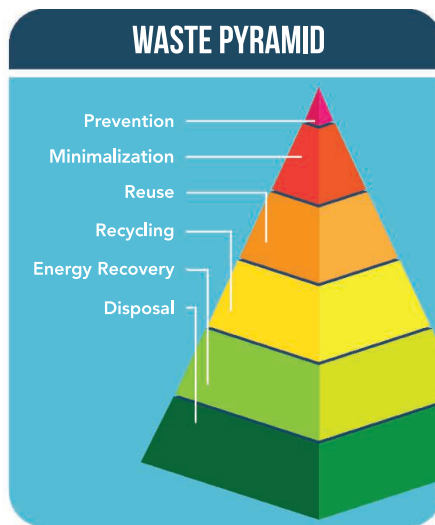
County, for example, and haulers are required to submit reports on what they pick up and where they take it. "We were swimming in data," he says. That's a relative rarity in the world of waste, where reliable statistics are often unavailable.

It may look convoluted, but Davidson's flow chart barely scratches the surface of the complexity, choices, and challenges that

modern society faces in managing its waste. For example, it deals with only a slice of the pie known as municipal solid waste (MSW). That's the highly visible trash generated by residents, schools, and businesses and picked up at the curbside or in parking lots. But MSW makes up only a tiny fraction—3% to 5% by weight is a good estimate—of the total waste that humanity generates.

The United States, for example, produces roughly 12 billion tons of waste each year, of which only 350 million tons are classified as MSW. The rest, sometimes called invisible waste, comes from mining, farming, road building and other construction, and industrial activities. There's also the human waste flushed down toilets and the pollutants dumped into waterways or spewed into the air (see infographics spread, p. 664).

In addition, the diagram only hints at the myriad contentious issues surrounding how waste is collected, processed, and ultimately disposed of in developed nations. Experts have varying views, for instance, on the best way to economically sift recyclables out of the MSW stream, the pros and cons of burning trash to produce electricity, and how to account for the hidden costs to a society of managing waste. Meanwhile, the once-radical idea of generating zero waste has shifted from the streets to the corporate boardrooms, sparking further debate over the extent to which such approaches will actually reduce global demand for important raw materials such as aluminum. The good news: Such discussions are prompting a closer look at what we throw away—and where it ends up.



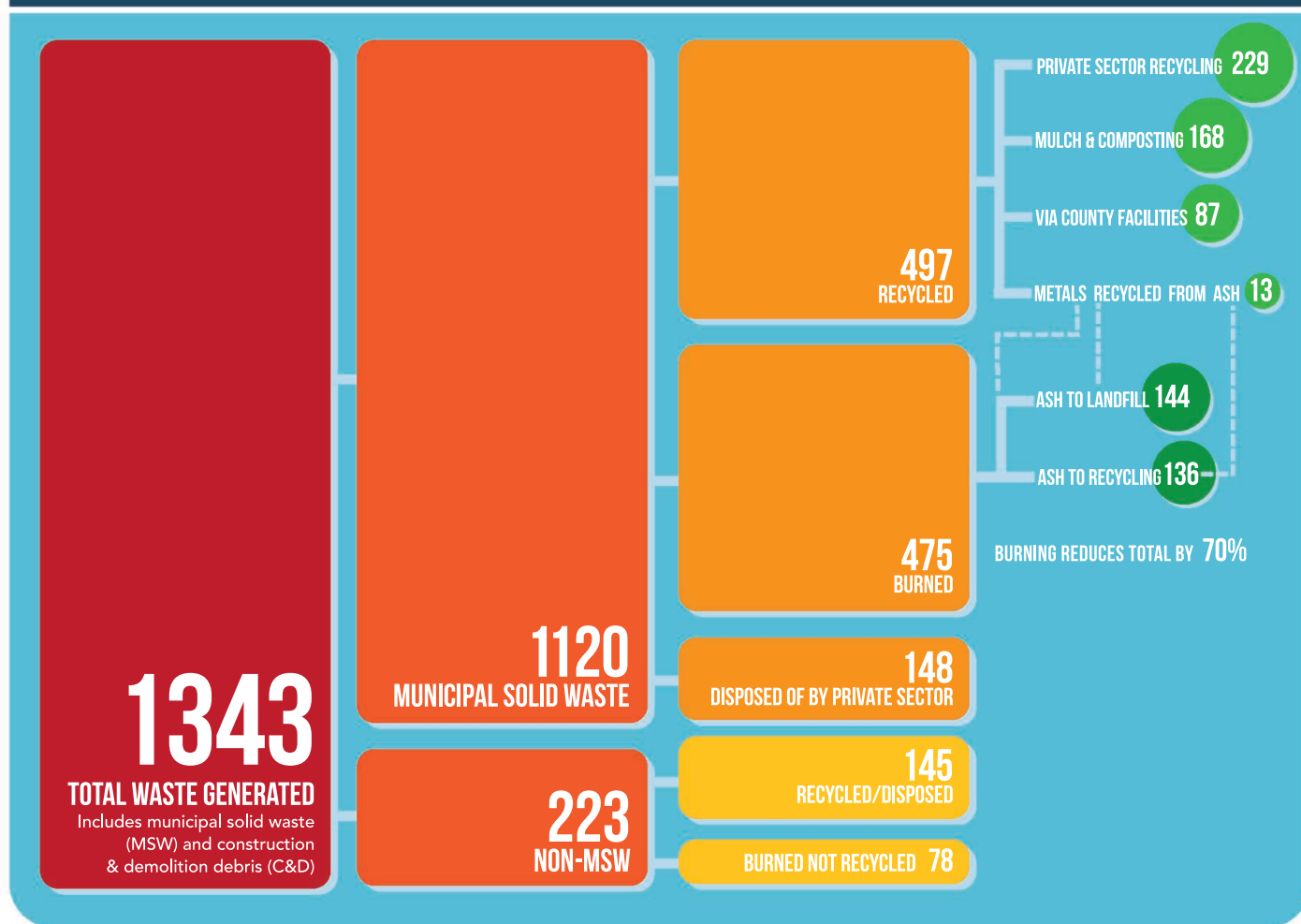
Shaping behavior. Waste professionals hope that humanity will eventually invert this pyramid and make waste reduction—creating little or none—the most popular option.

Bury, burn, and abandon

Trash wasn't always so complicated. "When I give talks on garbage, I start by saying our forefathers created waste with stone chips," says Wilson Hughes, former co-director of the Garbage Project at the University of Arizona in Tucson, which from 1973 to 2001 pioneered the science of garbology under the direction of urban archaeologist William Rathje. "But it didn't become a problem until they settled down and began living in one place. That's when societies had to start thinking about what to do with it."

Sitting around their fires at the end of the day, our ancestors had three choices for handling their waste, Hughes notes: "Bury it, burn it, or leave it on the floor." Fast-forward a dozen millennia, and those three options still exist. The only new wrinkle is recycling. (Of course, the idea of throwing out something that still possessed value might

INTRICATE STREAMS: THE FATE OF MONTGOMERY COUNTY'S WASTE (THOUSANDS OF TONS PER YEAR, 2011)



have seemed bizarre to our resource-starved ancestors, who reused bones, hides, and tools until they wore out.)

The choices a jurisdiction makes about how it handles its waste can have a big impact on its bottom line. “When Montgomery County needs to float a general obligation bond for a new school or road,” Davidson says, “it goes to New York to get its bonds rated. And the first question the bond companies ask is, ‘Have you got your solid waste act together?’”

Getting that act together can take time, however. Montgomery County, for example, got into the trash business by happenstance in the early 1940s. “Before then, your garbage was picked up by a guy with a truck” who probably took it to a local landfill, Davidson says. “And then World War II happened, the guy got drafted, and a [public] scream went up.”

Since then, local officials have developed what is now a comprehensive, integrated system. To start, the county requires residents and businesses to separate their waste into four streams—paper; plastics, metals, and glass; yard waste; and garbage. Private haulers working under county contracts then bring everything to a centrally located, county-owned transfer station and recycling center that also composts the yard waste. The recyclable material is sorted and stored on site, sometimes for months, until an appropriate buyer is found. The garbage is shipped a short distance by rail to an incinerator in Dickerson, Maryland, where it is burned; metals are then removed from the ash and recycled, and the remaining residue winds up in a landfill in central Virginia. The county currently operates no landfills, although it has a permit for a site near the Dickerson plant.

Burning debate

Although waste experts applaud Montgomery County’s overall approach, its reliance on incineration is more controversial. Landfills are the most common means of disposal for most of the United States outside the northeastern corridor and are especially popular in regions where land is relatively cheap and available.

Montgomery County’s incinerator was built and is operated by Covanta Energy, a New Jersey-based company with dozens of waste-to-energy plants across the United States. It’s called a resource-recovery facility because, unlike incinerators of the past, it operates with extensive pollution controls and separates and recycles metals after combustion. The 1800 tons of waste burned each day in its three boilers also generate 52 megawatts of electricity, which is sold to help offset operating costs.

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for sustainable waste management, and generating electricity is an added benefit," says James Regan, a corporate communications officer. A more complete life-cycle analysis, he says, would show that waste-to-energy plants actually reduce overall greenhouse gas emissions. They do that by diverting waste from landfills, which generate methane, and by reducing the amount of fossil fuels that must be burned in other plants to generate the same amount of electricity.

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The economics of waste handling also lie at the heart of another issue that is important to trash professionals: the scale, design, and business model used by recycling operations. Although almost anything can be recycled, experts note, market conditions often determine what is worth recycling.

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producing waste. But compared with those who toil in the steaming, vermin-infested mounds of garbage on the outskirts of Rio de Janeiro or Manila, Garcia and her crew work in relative comfort. They are provided with safety equipment, get regular breaks, earn well above minimum wage, and—although contract workers rather than regular county employees—receive the same health insurance. In fact, the regular hours and indoor venue make working the line a plum assignment and translate into very low employee turnover rates.

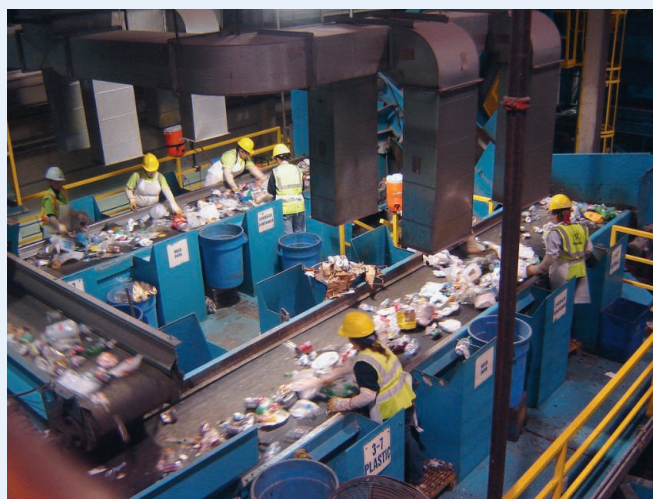
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—J.D.M.



Go with the flow. Line sorters at the recycling center.

a purer product can command a higher resale price. Toward that end, workers on the high-speed conveyor lines at the county's recycling center separate and bale more than a dozen different types of metals and plastics (glass is sorted into four categories but not baled), and the material may be kept for months until the county can find a buyer willing to pay a reasonable price. Montgomery County recycles 44% of its MSW, well above the national average of 24% but short of the county's own target, which it is in the process of raising from 50% to 70%. And what can't be sold must be disposed of.

Not far away, however, the behemoth of the trash industry, Waste Management Inc., operates a different model: It pays communities millions of dollars to bring their waste to its regional materials-recovery facility, and it doesn't demand that users sort their recyclables. For the company, economies of scale are the key to profitability.

During the week, a 24-hour stream of trucks arrives at the company's Kit Kat plant, which sits just off a major highway not far from the Baltimore/Washington International Airport. The trucks are delivering unsorted recyclable waste from customers throughout the mid-Atlantic region, including communities that, unlike Montgomery County, lack their own waste-handling facilities.

Kit Kat, which opened in 2007, can sort and process 75 tons of recyclable material an hour. (By comparison, Montgomery County's recycling facility handles 12 tons an hour and operates many fewer hours a week.) In 2010, Kit Kat processed 230,000 tons, 70% of it paper and cardboard.

Waste Management's business model depends on getting the best price possible for those bales of materials from buyers as close as Baltimore or as far away as Beijing. And unlike at a landfill or most transfer stations where the hauler pays a tipping fee to dump its load, Waste Management pays for what comes across its scales. "We do a sort test for each community," explains Jim Marcinko, head of the company's recycling operations for the Delmarva (Delaware, Maryland, and Virginia) area. "We'll analyze a whole day's worth of material. Then we'll deduct our processing fees and send them a big check." For Howard County, where Kit Kat is located, that check totaled \$3 million last year.

Waste Management's high-volume approach also gives the company a good reason to invest in potentially game-changing—and lucrative—new technologies. Currently,



Up in smoke. Cranes help manage the flow of waste to be burned at Covanta's Resource Recovery Facility in Dickerson, Maryland.

for example, most waste-to-energy facilities burn plastic to produce steam, which can be used to turn turbines to generate electricity. But that electricity has to compete in the market against relatively cheap sources of power such as coal and natural gas. In contrast, converting the plastic to higher-value transportation fuel could be a bigger moneymaker—if researchers can figure out a practical way to do it. That's why Waste Management is exploring the feasibility of using a high-tech version of pyrolysis, in which waste is heated to 2000°C in the absence of oxygen, to transform plastic into a substance that can be used as liquid transportation fuel.

Getting to zero waste

As Montgomery County and other municipalities grapple with the materials that end up in their recycling centers and transfer stations, some waste professionals would like researchers and companies to spend more time thinking about the front end of the waste stream, in other words, reducing how much trash we generate in the first place. That's the concept represented in a widely used "waste hierarchy," issued by the European Union in 2008, which depicts five options for dealing with trash (see graphic, p. 668). In descending order of preference, they are reduce, reuse, recycle, recover, and dispose.

Many in the "zero-waste" movement, in fact, see waste as the product of poor planning. "Waste is just a design flaw," asserts Montgomery County's Davidson. "If materials are created in such a way that they can't be recycled, then they need to be redesigned. And that's what we need to work on."

Garbologist Hughes, who spent a decade

managing waste-reduction efforts for the city of Tucson after leaving the university, says the real payoff from the zero-waste movement will be when companies begin "manufacturing things that can be taken apart and recycled. Stuff that has to be dumped won't be made any more."

General Motors (GM), the mammoth global carmaker, is one of many Fortune 500 companies that have embraced the concept of zero waste. In 2008, GM announced a goal of achieving "zero waste to landfill" at half of its 145 plants, a phrase that includes sending some of the company's waste to incinerators but that doesn't count the burial of ash residue. Two years later, it also promised to reduce the total waste generated at its facilities by 10% over the next decade. But what exactly do those targets mean for an automaker?

John Bradburn, an environmental engineer who has spent his entire 34-year career with GM and who now manages its waste-reduction efforts, says it means finding productive uses for material that

GM would have previously discarded. That includes making air-inlet panels from recycled bumpers, turning used packaging into sound-absorbing components within vehicles, and converting plastic waste into shipping containers. Bradburn says the initiative extends beyond the factory gates: The air deflectors on the Chevy Volt, GM's electric-gas hybrid car, were once oil-soaked plastic booms used to contain the 2010 *Deepwater Horizon* oil spill in the Gulf of Mexico.

Online

sciencemag.org

Podcast interview with author Jeffrey Mervis (http://scim.ag/pod_6095a).

At the same time, GM faces some constraints in reducing waste on the plant floor. “Vehicle parts must come to us in the best possible shape,” he says, “and to do that you need robust packaging” that creates additional waste and can be difficult to recycle.

GM says the zero-waste-to-landfill campaign is going smoothly, and an independent auditor recently verified its claims. As of June 2012, GM said 100 facilities—nearly 70%—have already achieved that goal. It hasn’t yet reported on progress toward its second goal—to reduce overall waste by 10% in the next decade—but GM says it did cut the amount of waste per vehicle manufactured by 28%, to 304 kilograms, in the 5 years prior to 2010.

Raw facts?

For advocates of sustainability, however, the real question is not what GM or any particular company is doing to reduce its waste. Instead, they want to know whether those steps are helping make significant progress toward a bigger goal: reducing the world’s demand for raw materials.

For one particularly valuable material, aluminum, a 2010 forecasting study by the U.S. Geological Survey (USGS) suggests that recycling is having less of an impact than might be expected. The issue involves how much of the projected rise in demand for aluminum—from 46 to 120 million metric tons—can be met with metal from so-called secondary sources, which includes recycled material. That estimate, in turn, rests on some fundamental assumptions about how aluminum is used. The heaviest demand in the next 2 decades will come from developing nations, the USGS report concludes. But those countries will tend to use aluminum mostly to construct long-lasting infrastructure such as buildings, bridges, and power lines.

As a consequence, the aluminum probably won’t be available for reuse for many decades, according to the report. In contrast, advanced economies tend to use aluminum in products with shorter lives, such as cars, trucks, and jets. Although most of that aluminum will be recycled, it won’t be enough to meet the global demand.

Based on that analysis, the USGS report concludes that “the proportion of aluminum generated from old scrap may decrease” between now and 2025. The industry disagrees, saying that there will be 50% more recycled metal available over the next 2 decades than the USGS has projected.

The dispute demonstrates just how hard



Boom times. Booms used to sop up the 2010 *Deepwater Horizon* oil spill have been combined with other plastics and recycled tires to make parts for the Chevy Volt.

it can be to measure the extent to which recycling helps conserve Earth’s resources. Some waste-management scholars worry that recycling has become a feel-good activity that diverts the public—and government agencies—from the need to find the most effective strategies to reduce waste and limit the unsustainable extraction of raw materials.

A new book by Samantha MacBride, an adjunct professor at Columbia University’s School of International and Public Affairs, argues that the primary burden of reducing waste should fall on the shoulders of manufacturers rather than on the public. “Recycling only affects 80 million tons of MSW a year [in the United States],” she notes. “It’s really only the tip of the iceberg.” Even so, the title of her book makes clear that she hasn’t abandoned all hope: *Recycling Reconsidered: The Present Failure and Future Promise of Environmental Action in the United States*.

Making the case for changing how the world perceives and handles waste, however, will require solid statistics. Without them, “trash talk” is too often simply that: uninformed opinions. But in the United States, at least, long-ago political decisions about how to regulate waste are limiting the flow of data.

In particular, twice within a decade Congress amended a potentially powerful tool to manage materials flow: the 1976 Resource Conservation and Recovery Act. The changes limited the U.S. Environmental Protection Agency’s ability to monitor and regulate large chunks of the U.S. waste stream. As a result, it now has responsibility for just two of the smaller pieces of the pie:

municipal and hazardous waste.

The outcome was predictable, says Sue Briggum, a longtime observer of federal environmental policy as vice president for federal public affairs at Waste Management. “If you only measure hazardous and municipal waste, that’s what will get managed and that’s what will get recycled. And what you don’t measure becomes invisible.”

Last year, EPA put out a notice asking for comments on whether it should expand the definition of MSW and look more broadly at what’s called “sustainable materials management.” Sensing a business opportunity, Waste Management told EPA that it welcomed the invitation to shift the discussion from “how to dispose of waste safely” to “the safe recovery of used materials.” But observers say federal legislators have little appetite for increasing the agency’s regulatory powers and, specifically, the scope of the law.

USGS, meanwhile, has proposed wiping out the small team of analysts who produce the federal government’s only comprehensive and authoritative studies on materials flows, including the report on aluminum. Agency officials admit that the \$5 million cut to the team’s parent office, part of a broader belt-tightening for 2013, “would reduce its ability to assist other federal agencies who rely on timely, accurate, and unbiased mineral resource data for decision making.”

In other words, eliminating the effort would make the government less able to manage its material resources wisely. Now that sounds like a real waste.

—JEFFREY MERVIS

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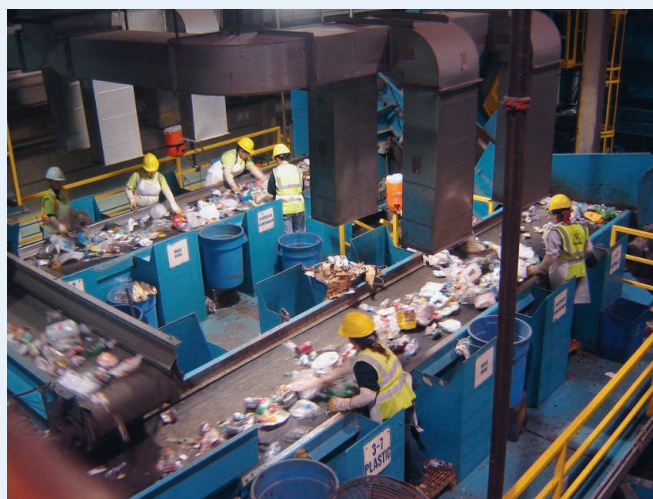
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FINDING A NEW WAY TO GO

The flush toilet was a transformative invention, but experts say its time may be past and are pioneering ways to recover energy and nutrients from human waste

At first glance, it's hard to see what the labs of stem cell researcher Michael Kallos and petroleum engineer Ian Gates could have in common. Excrement, however, has brought them together. For the past year, the University of Calgary scientists have been collaborating on inventing a new kind of toilet. Using their common expertise in designing bioreactors, they are devising a toilet that can convert feces and urine into fertilizer, clean water, and a source of gas for heating or electricity.

The world needs new toilets. The flush toilet “has provided great service and hygiene for 100 years or so,” says Arno Rosemarin, a sustainable sanitation expert at the Stockholm Environment Institute in Sweden. But it is undeniably wasteful. It uses drinkable water to carry away urine and feces, creating wastewater that has to be cleaned with expensive and energy-intensive technology (see p. 674). Flush toilets make even less sense in dry regions, and building sewage systems can be prohibitively expensive in the developing world. Even in wealthy regions, some existing systems don't treat waste adequately, discharging pollutants into downstream ecosystems.

Sanitation engineering, however, “hasn't been one of the most innovative sectors,” says M. Sohail Khan, professor of sustainable infrastructure at Loughborough University in the United Kingdom. That may be changing. Prompted by stricter clean water regulations and recognition that sanitation is key to improving public health, research into new ways of collecting and processing human waste is starting to catch on. Since 2010, for example, the Bill & Melinda Gates Foundation has awarded more than 50 grants on “next-generation sanitation,” including its “Reinvent the Toilet Challenge,” which aims to develop an attractive, eco- and user-friendly toilet that can process excrement for less than 5 cents per user per day.

One of the simplest alternatives to the “flush and forget” approach is the composting toilet, in which aerobic bacteria decompose waste. Given the right conditions, the composting process generates enough heat to kill dangerous pathogens, and with proper design, the toilets neither attract insects nor emit odors. Advocates have been tout-

ing composting's advantages for decades—one classic text is *The Humanure Handbook*, first published in 1995. Despite its advocates, however, the composting toilet hasn't caught on in private households outside a few “ecovillages” in Germany and in Swedish summer houses. That is partly due to psychological barriers (see p. 679), and partly because, at least in urban areas, someone needs to transport the finished compost away. (One solution to that problem is the Arborloo, in which the composting takes place in a pit dug under the toilet. When the pit is full, users move the toilet and plant a tree on the site.)

One promising concept for a “Toilet 2.0” is a design that keeps urine and feces separate. The bowl has two openings, one toward the front for urine and one toward the back for feces. Separation has several advantages, says Tove Larsen of the aquatic research institute Eawag in Dübendorf, Switzerland. Urine contains fewer pathogens than feces and so needs less intensive

S Video featuring the latest in toilet technologies.
<http://scim.ag/techtilet>

treatment to disinfect it. Urine also contains most of the nitrogen and much of the phosphorus that could be used as fertilizers. “If you have the nutrients in a concentrated solution, they are much easier to recover,” Larsen says. Finally, “dry” feces, not mixed with urine or large quantities of water, emit fewer odors, take up less space, and are easier to process either chemically or biologically. Drying feces is also an effective disinfection technique.

Larsen says separation toilets have promise for the developing world but sees potential in industrialized countries as well. Phosphorus is one of the main pollutants targeted

for removal by expensive water treatments. At the same time, it is a key component of fertilizer, and easily exploitable deposits are getting scarcer. But the element is relatively easy to recover from separated urine (compared to mixed sewage), Larsen notes. “We are perhaps a bit ahead of our time,” she says. “But at some point you won't be able to afford to put your urine in the nearest lake.”

With her toilet challenge grant, Larsen is working with Harald Gründl of the Austrian design firm EOOS to design a urine-diverting toilet that would recover clean water. In contrast, Kallos and Gates's toilet uses bacteria to digest mixed urine and feces anaerobically. It “in a way is an extension of your gut,” Kallos says. The digester will be a bit hotter, though—hot enough to sterilize the contents. Products include water, fertilizer, and methane that can be used for heating, cooking, or to produce electricity. Sohail's project uses hydrothermal carbonization, heating the waste under pressure to turn it into a coal slurry. They recover water from the mix and burn the coal to power the system.

The energy potential of feces is one reason the Gates Foundation launched the toilet challenge, says Doulaye Kone, a senior program officer at the philanthropy. “There's enough energy left [in digested food] to heat something, to drive something,” he says. “If you could harness that, you could invent a standalone toilet that could bypass the sewer.”

On 14 August, the first eight challenge research teams will gather in Seattle, Washington, to demonstrate their prototype toilets. The foundation will then choose one or two designs to develop further. So far, Kone says, “the portfolio is very, very encouraging.” Stay tuned for Toilet 2.0.

—GRETCHEN VOGEL



Going green. A toilet that separates urine and feces makes it easier to recover nutrients and clean water.



Saving energy. Washington, D.C.'s wastewater treatment plant is making changes to become more energy efficient by converting sludge to methane.

ble the average flow of the Nile), and that they have helped dramatically improve water quality and reduce disease.

Increasingly, however, water-reclamation engineers are under pressure to do even more. One good place to see this evolution in action is the Blue Plains Advanced Wastewater Treatment Plant in Washington, D.C. The sprawling 75-year-old plant, which serves more than 2 million residents in the Washington, D.C., region and processes some 1.4 million cubic meters of wastewater daily, is the biggest of its kind in the world. And over the next decade, it will spend \$1 billion implementing new approaches for coping with two major problems common to many plants:

dealing with tainted sludge and removing nitrogen from the waste stream.

WATER RECLAMATION GOING GREEN

Wastewater treatment plants are revamping themselves as resource recovery operations, saving—and even producing—energy and polluting less in the process

“There is no such thing as waste, only wasted resources,” says Chris Peot, a civil engineer with DC Water, the utility that treats wastewater for the Washington, D.C., region. And with that as his motto, Peot has become a foot soldier in a revolution sweeping across the world’s wastewater treatment plants.

Gone are the days when it was good enough to remove fecal matter, urine, and bacteria from wastewater and dump the resulting sludge into a landfill while pouring the cleansed water into the nearest river or ocean. Over the past few years, sewage treatment plants in industrialized nations have begun to reinvent themselves, their operators motivated by tighter regulations and economics to put the byproducts of treatment processes to good use. Aided by new technologies and old technologies put together in new ways, the facilities are becoming more efficient, cutting energy use and—in an increasing number of cases—producing more energy than they need to operate. And with the help of a common,

but until recently underappreciated, bacterium, they are getting rid of pollutants for less cost (see sidebar). Treatment plants “are becoming the water resource factories of the future,” says Amit Pramanik, an environmental engineer with the Water Environment Research Foundation in Alexandria, Virginia, a nonprofit organization that funds research in wastewater treatment.

Trendsetter

Since engineers designed the first rudimentary systems for treating sewage with chemicals or sand filters in the late 1800s, specialized facilities for handling human waste have grown more common and complex. Now, there are more than 400,000 centralized sewage treatment plants around the world. Although good statistics are scarce, some experts estimate that altogether, wastewater treatment plants process more than 730 million cubic meters of wastewater daily (more than dou-

Sludge for energy

Sludge—a goopy, mudlike mix of organic matter and dead microorganisms—is a byproduct created at almost every step of the treatment process. When sewage flows into Blue Plains and most other treatment plants, for example, it first goes through a settling stage called primary treatment that allows suspended solids to sink to the bottom of large tanks. Then, the water undergoes secondary treatment, in which microbes process food and fecal waste, creating more sludge. At many plants, the liquid effluent is returned to a local river or ocean after secondary treatment. But at Blue Plains and other plants that discharge into sensitive waterways, a third tertiary treatment is added to remove nitrogen and phosphorus compounds. All told, Blue Plains’ three-step process produces some 1200 tons of sludge, or “biosolids,” every day, enough to fill 50 tractor-trailers.

Historically, treatment plants simply dumped their sludge into landfills or treated it with lime and spread it on farm fields. Such practices have drawn criticism, however, because transporting sludge is expensive and dumping it wastes

S Video featuring a trip to the wastewater treatment plant.
<http://scim.ag/wastetx>

A BETTER WAY TO DENITRIFY WASTEWATER

Call it the case of the missing nitrogen. Forty years ago, wastewater treatment engineers noticed that a common process used to convert ammonia into nitrate sometimes failed to produce as much nitrate as expected. The nitrogen “must have gone somewhere,” says Mark van Loosdrecht, an environmental engineer at the Delft University of Technology in the Netherlands. Fermentation engineers determined that the process was producing nitrogen gas, but nobody knew how.

Then, in the early 1990s, microbiologist Gijs Kuenen of Delft University and his colleagues discovered a new microbe in wastewater that helped solve the mystery—and turned existing dogma about ammonia’s conversion to nitrogen compounds on its ear. Called anammox (for anaerobic ammonium oxidation), the microbe was converting ammonia into nitrogen gas in the absence of oxygen, a reaction previously thought impossible.

It took several years to convince the skeptics. One problem was that the bacterium—which is in the phylum *Planctomycetes*—grows slowly. It divides every 2 weeks, rather than in just half an hour like some bacteria; that means it can take months and sometimes years to get a culture up and running reliably in the laboratory. Another challenge was that the bacteria had never been found in the wild. Once researchers knew what to look for, however, they found it and its relatives living in many places—in oxygen-poor waters of the Black Sea, Lake Tanganyika, and off the coast of Namibia, for example.

Now, researchers consider anammox bacteria to be essential components of the global nitrogen cycle and estimate that they account for 50% of the world’s nitrogen turnover. And they believe the microbes could dramatically improve methods of removing ammonia from wastewater streams at large municipal plants like the Blue Plains treatment facility in Washington, D.C. (see main text). “It’s possibly going to be a game-changer in the U.S.,” says Kartik Chandran, an environmental engineer at Columbia University.

Harnessing anammox’s potential, however, requires a mastery of microbial ecology. The microbe must be grown in conjunction with a second bacterium that converts ammonia to nitrite; anammox converts the nitrite into water and nitrogen gas. But to operate efficiently, the system must also exclude bacteria that make nitrate. That’s proven relatively easy in industrial

processes that operate at high temperatures and produce relatively warm, ammonia-rich wastewater streams; several companies have already commercialized anammox systems for use in such environments.

But excluding nitrate producers has proved harder in lower-temperature municipal wastewater treatment plants, where the concentration of ammonia can also be low, says van Loosdrecht. Under those conditions, it’s been tricky to create a stable anammox community, although a number of plants have installed pilot anammox, also called deammonification, systems.

To solve that problem, van Loosdrecht has been experimenting with very slow-growing anammox microbes. Typically, dividing bacteria form suspended particles called floc. But these slow-growers form a much larger, denser particle called a granule. The larger granules somehow tend to exclude the nitrate-producing bacteria. To take advantage of that characteristic, he’s engineering a reactor that retains larger granules but excludes smaller floc; he predicts the reactors will enable treatment plants to “do the same process [with] 25% of the space” used by current systems, and cut energy and other costs by about one-third.

Columbia’s Chandran, who once isolated a strain of anammox bacteria from a Brooklyn, New York, treatment plant and now has it happily growing in his lab, is also perfecting ways to keep the microbe happy and healthy in wastewater treatment plants. Since 2010, treatment plants developing anammox systems have been sending him samples weekly, or more often if they suspect problems. Drawing on findings from his research, he tests the health of a plant’s anammox community by sequencing the DNA that covers the microbes’ 16S ribosomal subunits. Each type of microbe has a unique 16S fingerprint, and he can tell what kind and how many anammox organisms are present by the number of copies of the 16S genes. His team also looks at the expression of the microbe’s key ammonia-fixing genes by monitoring messenger RNA. If Chandran sees 16S numbers and gene activity dropping, he knows the system needs tweaking—there might be too much oxygen, for example. If gene activity is dropping, but the population is stable, it’s likely to be a transient phenomenon that should right itself, he says.

Such efforts are nudging deammonification into more widespread use. “There’s no scientific limitation,” van Loosdrecht says. “It’s purely an engineering question.”

—E.P.



Going red. With this cone, engineers gauge the density of the clumps of red anammox bacteria (*inset*), a new way to denitrify wastewater.

a potential source of energy. In response to such concerns, Blue Plains is installing technologies that will enable it to convert one-half of its sludge into methane for use as fuel, with the remainder processed into a high-quality, pathogen-free material that could be used like compost in landscaping.

The heart of that new system is currently a construction site for a new building where

biosolids will be subjected to high pressure and heat—150°C for 30 minutes. During this pasteurizing process, known as thermal hydrolysis, bacterial cells in the sludge will burst, making them more amenable to being eaten by methanogens, microbes that produce methane gas that can be used for fuel. The fuelmaking process will occur in huge new tanks called digesters, and the meth-

ane in turn will be used to fuel a turbine that will produce electricity and heat that will power the thermal hydrolysis system and net enough extra electricity to power 8000 homes. The remaining pasteurized biosolids, meanwhile, will be available for use as fertilizer. Adding thermal hydrolysis to the system saved about \$200 million in digester construction costs because the process con-

centrates the cells and less space is needed for methane conversion, Peot says. “Without that [amount of savings] we would not have been able to afford the digesters,” he says.

Waste streamlined

A second Blue Plains project, being undertaken in conjunction with two other treatment plants in Austria and Virginia, is coming up with a better way to remove nitrogen compounds from wastewater. Ironically, part of the nitrogen problem is created by the methane-producing digesters; the microbes release a lot of ammonia into the wastewater stream. At first, Peot and his colleagues considered taking that water and putting it through the entire treatment process again to remove the ammonia. But that multistep process, which involves aerobic bacteria and methanol, is costly, in part because sustaining the bacteria requires aerating, or adding oxygen, to the water, which accounts for almost one-quarter of the Blue Plains plant’s power use. The process also yields carbon dioxide, increasing the plant’s carbon footprint.

Instead, they decided to use an increasingly popular bacterial process, called anammox or deammonification, to get rid of the nitrogen compounds (*Science*, 7 May 2010, p. 702). To start with, Blue Plains will use anammox on only the ammonia-rich water coming out of the digesters. But if the process works as advertised, Peot envisions using it for the entire waste stream. And “if we are successful,” he says, Blue Plains will cut its power use for aeration by two-thirds and, together with other energy-saving measures, almost get by on just the power generated using its own methane. “Our goal is to investigate ways to become energy neutral or even energy positive,” he says.

Other plants are finding that reaching that goal is possible. In 2004, for example, anammox combined with other measures enabled a plant in Strass, Austria, to become energy self-sufficient. A similar multifaceted approach is enabling the East Bay Municipal Utility District, which serves part of the greater San Francisco area in California, to use various types of organic waste, including chicken blood and cheese waste, to generate enough power to run its treatment process as well as 13,000 homes. As the East Bay’s director of wastewater, David Williams, puts it, “We’ve turned wastes into commodities.” It’s an achievement Blue Plains and other waste-treatment plants hope to emulate.

—ELIZABETH PENNISI

PAVE SAVE THE WORLD!

Carbon dioxide may be the ultimate industrial waste product. Could tropical corals provide a trick for locking it away in our highways?



Researchers who think about how best to stave off the worst impacts of climate change often have their favorite way of disposing of one prominent industrial waste product: carbon dioxide (CO₂). Some urge planting trees to soak up the greenhouse gas; others say capture it and pump it underground. For Brent Constantz, the solution is pavement, and lots of it.

Drawing on the chemistry that corals use to build their rock-hard shells, the California entrepreneur and biomineralization expert hopes to combine simple seawater with CO₂

to manufacture cement and concrete that would devour vast amounts of the greenhouse gas. “I honestly don’t think we can address the carbon problem any other way,” says Constantz, a consulting associate professor at Stanford University in Palo Alto, California.

It’s an audacious idea he’s already tried to commercialize once—with mixed results and plenty of criticism. And his peers are divided on the prospects for seawater cement. “Brent has been too optimistic about these kinds of processes in the past and too glib about the challenges,” says Roger

Concrete plans. Brent Constantz hopes to use advances in biotechnology to make cement the way coral do and in the process lock up billions of tons of CO₂ before it can reach the atmosphere.

Aines, a geochemist at Lawrence Livermore National Laboratory in California. But Constantz is “a brilliant man” with “a very interesting idea,” says Barry Blackwell, president and CEO of Akermin, a start-up in St. Louis, Missouri, that also works with CO₂.

Abundant waste

There is certainly plenty of work needed to deal with humanity’s most abundant waste product. In simple numbers, burning fossil fuels generates nearly 35 billion metric tons of CO₂ per year. Roughly 40% of that CO₂ comes from some 5000 power plants that burn coal and natural gas to produce electricity.

The likely impact of that waste is well known. Since 1750, the amount of CO₂ in the atmosphere has risen from 280 to nearly 400 parts per million (ppm) today. By 2100 it’s expected to reach 500 to 1000 ppm, depending on progress in cutting emissions. That’s expected to lock in a global average temperature increase of up to 5.2°C, trigger sea-level rise, and boost ocean acidity by as much as 150%.

The world won’t have a prayer of limiting the CO₂ buildup unless nations find ways to either prevent or lock up billions of tons of CO₂ emissions per year. Constantz, for one, doesn’t see many ways to prevent emissions quickly at that large of a scale. Although he says it is critical to improve energy efficiency and develop low-carbon energy sources—such as wind and solar—he doesn’t think that will be enough. And he believes that carbon capture and storage technologies—separating carbon dioxide from industrial emissions, liquefying it, and injecting it underground—will be too costly to be widely adopted.

If researchers can develop a valuable product that gobbles up CO₂ during manufacture, however, that would dramatically lower the cost barrier to megascale carbon capture, he says. And for Constantz, the most obvious candidates are cement, concrete, and the mix of sand and gravel known as aggregate, all of which are used to build infrastructure, from bridges to buildings. The world today produces some 2.5 billion tons of Portland cement annually, for example, as well as 12.5 billion tons of concrete and 32 billion tons of aggregate. Using those mountains of material to trap



Copycat. Marine corals, such as this reef in the North Pacific, inspired the idea for seawater cement.

carbon is “a properly scaled solution to the problem,” Constantz says.

To achieve that solution, however, Constantz needs to turn the current concretemaking process upside-down. Today, manufacturing the materials actually produces—rather than traps—huge quantities of CO₂. Making a ton of concrete, for example, generates a ton of CO₂. Overall, in fact, concrete production accounts for about 5% of all carbon emissions globally.

Tropical inspiration

Constantz, 53, says the idea of transforming concrete from a carbon source into a carbon sink came from his early academic work studying how tropical corals make their shells. But he quickly turned to the corporate world. When he was 27, he used his experience studying marine calcification to come up with and commercialize a novel cement that revolutionized the repair of bone fractures in hospitals around the globe. In 2002, he revised his recipes to develop a stronger and faster-setting cement. He now holds nearly 100 patents in cement-making technology.

As he mixed his boutique medical cements, however, Constantz began to wonder whether it might be possible to turn

waste CO₂ into the minerals that form the basis of structural cement and concrete. In principle, the idea is straightforward. When dissolved in water, CO₂ reacts with water to make bicarbonate ions, which under the right circumstances are transformed into carbonate ions. When carbonate meets up with calcium and magnesium ions present in seawater, they readily combine to form calcium and magnesium carbonates; those form the same minerals used by many marine organisms to build their shells, as well as what is found in standard industrial Portland cement.

In 2007, Constantz got the chance to turn his idea into a company called Calera. Backed by Khosla Ventures, a Silicon Valley venture-capital firm, it raised \$182 million to build a pilot cement plant next to a power station in Moss Landing, on the California coast. A pipe from the plant carried CO₂-rich flue gas into the base of a 33.5-meter-high tower. The gas then streamed up through the tower, where it met droplets of seawater raining down. Calcium and magnesium carbonates formed as the gas and water

collided and then sifted down in a fine white powder that was dried and used to make cement. Instead of producing CO₂, however, the company said the process actually sequestered one-half ton of CO₂ per ton of concrete. The upshot, they argued, was that humanity could solve its CO₂ problem by using seawater cement to build more roads and buildings.

Outsiders, however, were far from convinced. Some critics argued that the company was too secretive about its process, and that something else must be going on for the chemistry to work. One outspoken critic was Ken Caldeira, a climate scientist at the Carnegie Institution for Science at Stanford University. Caldeira argued that getting calcium and magnesium ions to bind with CO₂ to precipitate out of water isn’t easy. That’s because carbonate ions are stable in water only if the pH is above 9.5 (far more alkaline than the 8.1 pH value of seawater). In natural seawater, the lower pH causes carbonate ions to pick up an extra H⁺ to become bicarbonate. Many shell-building organisms get around this by creating microenvironments with high pH to precipitate out their needed minerals.

Caldeira was right. It turned out Calera engineers were adding sodium hydroxide or other strong bases to their seawater to make

it more alkaline, driving the pH as high as 12 or 13. The higher pH allows more CO_2 to dissolve into the water and then speeds the precipitation of calcium and magnesium carbonates. The problem is that alkalinity isn't free. And although the Moss Landing site had a ready source of alkalinity—piles of magnesium hydroxide left over from the plant's former life of making metal for World War II bombs—most power plants do not (although Constantz estimates that 10% of coal and natural gas power plants worldwide are located next to plentiful sources of alkalinity).

In the end, despite early demonstration successes, Calera's cementmaking process turned out to be too expensive to be broadly applicable. And last year, the company replaced Constantz as president, shifting its focus to using the process to make other, higher-value chemicals. "That's not why I founded Calera," Constantz says of the specialty chemicals business. "I founded it to sequester gigaton levels of CO_2 ."

A second try

Now, Constantz is back at Stanford University, working on what he calls his generation-2 approach. Like the earlier effort, Constantz envisions bubbling CO_2 into seawater to bind it into solid minerals. But this time he hopes to run the process with a gentler and, he hopes, cheaper chemistry.

The key, Constantz now believes, is carbonic anhydrase (CA), an enzyme that whisks CO_2 out of our blood and into our lungs to be exhaled. Corals use their own version of CA to pull CO_2 out of seawater to build their shells. This process happens slowly without the enzyme. But CA speeds it up as much as 1 million-fold. And speed is the key for industrial reactions. A fast reaction, for instance, could dramatically reduce the size of the tower used to mix water and CO_2 . Instead of a 33.5-meter tower, for example, the process might need one just 3.5 meters high. That, Constantz hopes, will significantly reduce the capital costs of setting up seawater cement plants.

Speeding up the reaction might also help reduce the need to add so much alkalinity. Even at a pH of 9.5 without CA, few bicarbonate ions turn into carbonate and become available to bind with calcium and magnesium to form calcium and magnesium carbonates. That's why Calera was forced to add powerful bases, to pump the pH up above 12

and accelerate the reaction. The addition of CA could change all that.

Most CAs have a zinc atom at their core surrounded by four amino acids called histidines. The histidines help zinc bind to a water molecule. Once it does, the protein's core essentially splits water (H_2O) into a hydroxyl group (OH^-) and a proton (H^+). The hydroxyl quickly binds with CO_2 , forming bicarbonate (HCO_3^-), and the H^+ floats off into solution. The fastest CAs can perform this little molecular dance 1 million times a second, fast enough to turn lots of CO_2 into bicarbonate, which then goes on to form carbonate if the pH is 9.5 or higher.

But there's a catch. "For this reaction to continue, you need to maintain this pH," says Alex Zaks, a biochemist and Akermis's chief technology officer. But if H^+ ions continue

it to work in an industrial setting. For starters, CAs are fragile proteins that fall apart in hours to days at about 40°C , well below the temperature of a normal power plant flue gas. Some companies have already taken steps to make them hardier. Researchers at Akermis, for example, have shown that by encapsulating a CA variant in a CO_2 -permeable polymer, they can keep their enzyme stable and highly active for at least 90 days even above 40°C . Aines and his colleagues, meanwhile, reported online 6 June in *Inorganic Chemistry* that they've made a synthetic CA mimic that withstands temperatures up to 100°C . Although the catalytic activity of the mimic is more than 10-fold slower than natural CA, Aines says his team is now working on versions that are faster. Finally, Codexis, a Redwood City, California-based company,

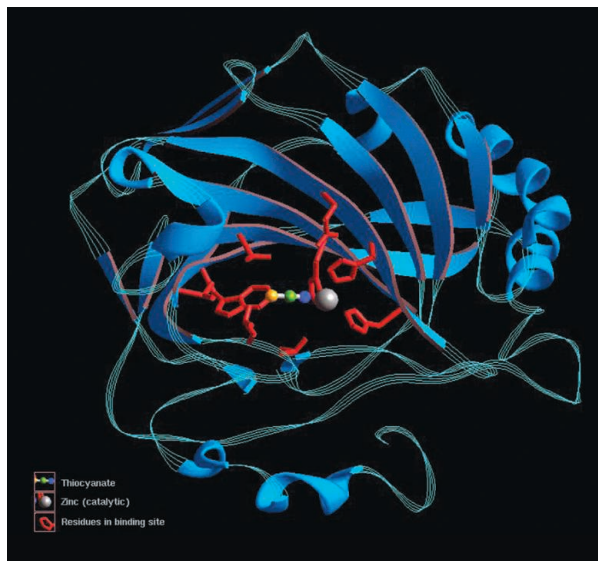
reported last month that one of their engineered CAs remained stable during a field test at a coal plant in which temperatures reached as high as 82°C .

Other companies are also exploring CA to make seawater cement. Last year, for example, Quebec City, Canada-based CO_2 Solutions teamed up with Codexis and aluminum maker Alcoa to pursue a similar strategy. However, last month the companies announced that they put their pilot project on hold because they couldn't meet their timeline from the U.S. Department of Energy, which was funding much of the work.

That means, for now, most companies working on CA are looking at using it to develop a cheaper method of purifying CO_2 from flue gases. Their hope is either to pump the purified CO_2 into old oil wells to push out additional oil, or to feed it to algae to produce plant oils that can be converted into transportation fuel.

Such work underscores that Constantz is not alone in his hope to use CA to convert billions of tons a year of CO_2 waste into something valuable. And for now, Constantz's dream of using CA to pave his way to saving the world remains just that. But geologist Gordon Brown, a Stanford colleague, says people would be premature to dismiss Constantz. "He's often prescient and sees things before others do," he says. And anyone hoping to curb climate change is probably hoping Constantz's dream comes true.

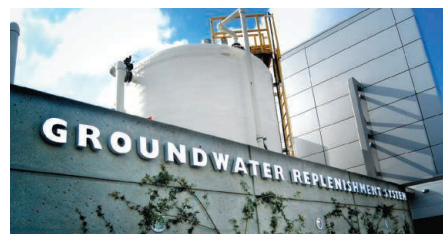
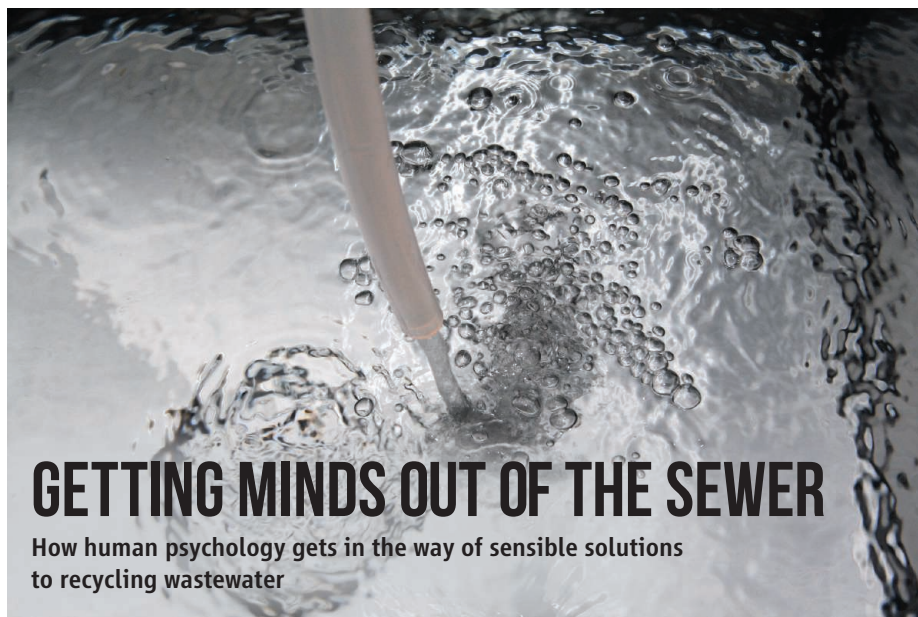
—ROBERT F. SERVICE



Take two. By adding the protein carbonic anhydrase, researchers hope to make seawater cement more affordable.

to stream into the seawater, they will steadily drive down the pH, increasing the water's acidity (pH is the measure of available H^+ ions in solution). And if the pH drops too low, only bicarbonate will be stable in solution, not carbonate. That means even with CA, engineers will still have to add chemical bases continually. But Constantz says he's hopeful that CA will enable them to use less strong, and less expensive, bases such as coal ash, a waste product from coal-fired electric plants. "The logic does make sense," Zaks says. However, until he sees a working demonstration plant and the numbers to go with it, he's remaining noncommittal.

Constantz and others will also need to make other improvements to CA to enable



In water-starved Orange County, California, engineers take treated wastewater from sewage processing plants and put it through a battery of filters and purifiers that produce a fluid that is more than clean enough to drink, according to government standards. A county facility opened in 2008 churns out 70 million gallons of this recycled water a day, enough to meet the needs of 600,000 residents. But instead of piping the ultrapure water to people's kitchen sinks, the county pumps it into the ground.

The reason has more to do with psychology than with engineering. The public was too squeamish about drinking recycled wastewater straight from the tap, local officials say; the "yuck factor" was just too great. So they came up with an alternative: About half of the reclaimed water is injected into wells to prevent seawater from seeping into local aquifers; the other half goes into basins, where it filters through sand and gravel to replenish the aquifers that supply drinking water. "This perception of a natural barrier where it's blending and mixing with all of our other water supplies ... helps people make the leap," says Eleanor Torres, director of public affairs for the Orange County Water District and its euphemistically named Groundwater Replenishment System.

Technologically speaking, it's no huge feat to turn water contaminated with human waste into a usable resource. A report earlier this year by the National Research Council of the U.S. National Academies found that wastewater reuse could provide up to 27% of the public water supply in coastal commu-

nities in the United States. But getting communities to accept such projects often isn't easy. That's because—whatever the science says—winning people over involves the delicate work of overcoming deep-seated psychological barriers and cultural taboos surrounding human waste.

Cognitive sewage

The aversion to excrement is deeply rooted in the human psyche, and for the most part it serves us well, says Valerie Curtis, an evolutionary psychologist at the London School of Hygiene and Tropical Medicine. For our human and prehuman ancestors, pathogens were probably a greater overall threat than predators, Curtis says. That's why we have a strong, intuitive sense of disgust, she says: "Pretty much all the things we find disgusting have some kind of connection to infectious disease."

Those intuitions can easily trump reason, says Paul Rozin, a psychologist at the University of Pennsylvania and a pioneer of research on disgust. In one classic experiment in the 1980s, Rozin gave college students a piece of fudge shaped like a dog turd. "They know it's chocolate, okay, and they like chocolate, but most of them won't eat it," he says.

In fact, disgust can evoke what Rozin and colleagues call "magical" thinking. In one demonstration of this, they presented undergraduate students with a glass of juice. Then, using forceps, a researcher dipped a dead, sterilized cockroach into the glass. Despite

Drink up. California Representative Loretta Sanchez (D) (*above*) demonstrates the drinkability of recycled water at the inauguration of a treatment facility in Orange County.

assurances that the juice was perfectly clean and safe (which it was), the students had a strong aversion to taking a sip. And it didn't stop there. Even when the researchers provided new glasses filled with juice, students still didn't want to drink. It was as if people believed that the newly poured juice had somehow been contaminated by the roach, says Rozin's then-graduate student, Carol Nemeroff, who is now at the University of Southern Maine, Portland. Nemeroff thinks the same logic-defying thought process comes into play in getting people to accept recycled wastewater, especially for drinking. The question, she says is: "How do you get the cognitive sewage out, after the actual sewage is gone?"

Sometimes you can't. The yuck factor has scuttled proposed wastewater recycling projects in San Diego, Los Angeles, and elsewhere. Opponents of these projects effectively used slogans like "toilet to tap" to create a stigma that's hard to overcome, says Paul Slovic, a psychologist at the University of Oregon, Eugene, and president of Decision Research, a nonprofit research organization. Since the 1990s, Slovic has studied the mental shortcuts people use to assess risk. In work on attitudes toward nuclear and chemical waste disposal, for example, he found that whereas experts methodically tote up

Online

sciencemag.org

Podcast interview with author Greg Miller (http://scim.ag/pod_6095b).

risks and benefits, laypeople tend to decide intuitively whether a given material or technology is either good or bad. And once they decide a technology is bad, they tend to overestimate the risks and downplay the benefits. “For most of us, risk perception is not the output of a scientific, mathematical calculation, but of a gut feeling,” Slovic says.

Let's be reasonable

Water agencies have taken note of this research, and in some cases they're commissioning studies of their own. Slovic, Rozin, and Nemeroff collaborated on a 2008 survey of public attitudes sponsored by WaterReuse, a nonprofit organization in Alexandria, Virginia. That study, led by Brent Haddad, a social scientist at the University of California, Santa Cruz, found that educating consumers about the water cycle can help increase acceptance of recycled water. Most people have no clue what happens when they flush the toilet, Haddad says. In most of the developed world, what happens is this: Collected wastewater is treated to remove solids and pathogens and then pumped into the nearest natural body of water, where it can enter the water supply of the next community downstream. “Any city that's at the bottom of a river is drinking the recycled wastewater of cities upstream,” he says. His survey found that when people realize they've been drinking unintentionally recycled water, they're more willing to accept intentionally recycled water.

A more recent study sponsored by WaterReuse expands on this idea, suggesting that framing reuse projects in the context of the urban water cycle—in which all water is essentially recycled—can help make them more acceptable. The focus should be on what the water is now (clean and safe) rather than where it came from in the recent past (a sewage treatment plant), says Linda Macpherson, a co-author of the recent study and a reuse technologist at CH2M Hill, an engineering and consulting firm that works with water agencies around the world. “We've got to get people to start thinking of water as a reusable resource,” Macpherson says.

Borrowing a few lessons from psychology might help water-reuse projects gain traction, but it takes old-fashioned politicking too. In Orange County, the water district made sure key politicians were onboard from the beginning, and they reached out to various communities they knew were likely to be wary of the project, including mothers' groups and the region's Vietnamese and Latino immigrant communities, which have tended to be sus-



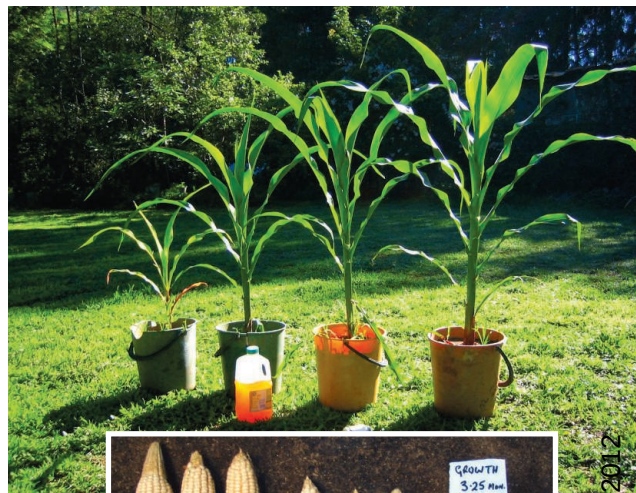
Brown and yellow revolution. Composted feces (upper left) and urine (upper right) can increase crop yields, if the intuitive aversion to handling human waste can be overcome.

picious of the government, Torres says. The district tried to build trust in those communities by sending local doctors and engineers to answer their concerns. And they're doing it again now to pave the way for an expansion of the Groundwater Replenishment System that will increase its capacity to 100 million gallons a day by 2014. Torres thinks acceptance is growing and will eventually lead to direct potable reuse. “The younger generation, I think they get it more,” she says.

The poo taboo

Disgust for feces is universal, but it varies in degree in different cultures, says Sarah Jewitt, a geographer at the University of Nottingham, University Park, in the United Kingdom. In China and other parts of Southeast Asia, for example, people have used human manure to fertilize crops for centuries, Jewitt says. China is also a leader in biogas production from human and animal feces. “I would describe them as a more fecophilic society,” Jewitt says. “They have fewer taboos.” Indian society, in contrast, is one of the more fecophobic. That manifests in a number of ways, including the stigma faced by the workers who clean toilets and remove waste from community latrines, Jewitt says.

Attitudes also tend to fluctuate with time. Jewitt notes that the editorial pages of British newspapers in the 1840s and 1850s endorsed collecting London's sewage to fertilize nearby farms. By the end of the Victorian era a few decades later, however, enthusiasm for such endeavors dimmed and the flush-it-and-forget-it men-



tality became predominant, Jewitt says.

But in some European circles, the pendulum is swinging back. Composting toilets and diversion toilets that collect urine for use as fertilizer (see p. 673) have been widely adopted by some communities in Germany and Sweden. These so-called ecological sanitation (“ecosan”) technologies could be especially beneficial in parts of the developing world where water is scarce and no sewage infrastructure exists, says Elisabeth von Muench, who oversees ecosan efforts for the German Agency for International Cooperation. Ecosan advocates would like to see people in developing countries leapfrog flush toilets, just as they've gone straight to mobile phones without a landline stage. But composting toilets haven't yet taken off on a global scale—at least partly, experts believe, because the small amount of hands-on upkeep required can run headlong into taboos about handling excrement.

In general, the places where ecological sanitation, wastewater recycling, and other alternative strategies for handling human waste have taken root are those where the waste can be used to fulfill an urgent local need: for fertilizer, energy, sanitation, or clean water. In other words, these projects tend to have the best chance of success in places where intuitive disgust can be overcome by other powerful forces of human psychology—such as the desire to live a healthier, wealthier, and more comfortable life.

—GREG MILLER

REVIEW

Taking the “Waste” Out of “Wastewater” for Human Water Security and Ecosystem Sustainability

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Humans create vast quantities of wastewater through inefficiencies and poor management of water systems. The wasting of water poses sustainability challenges, depletes energy reserves, and undermines human water security and ecosystem health. Here we review emerging approaches for reusing wastewater and minimizing its generation. These complementary options make the most of scarce freshwater resources, serve the varying water needs of both developed and developing countries, and confer a variety of environmental benefits. Their widespread adoption will require changing how freshwater is sourced, used, managed, and priced.

More than 4 billion people live in parts of the world where freshwater scarcity directly threatens human water security or river biodiversity (1). Threats to human water security can be overcome by building centralized infrastructure that harvests, stores, treats, and transports water for agricultural, industrial, and municipal uses. For countries that can afford it, this approach has greatly benefited human health and economic development, but it is often energy-intensive and comes at a steep ecological price. In the developing world, on the other hand, an estimated 1 billion people lack access to safe affordable drinking water, 2.7 billion lack access to sanitation, and many millions die each year from preventable waterborne dis-

eases (2). Thus, developed and developing countries face separate but overlapping challenges. In developed countries, existing water infrastructure needs reengineering to sustain a high standard of living while reducing its environmental footprint and sustaining or restoring biodiversity. In developing countries, affordable infrastructure is needed to satisfy the water needs of humans and to preserve aquatic ecosystems (1). Meeting these twin challenges will require striking a balance between delivering new sources of water and using water more productively through pricing, conservation, and wastewater reuse.

How Is Water Used and Wasted?

Water use can be classified as consumptive or nonconsumptive, depending on how readily the used water can be reused. Consumptive use converts water into a form that cannot be reused. A portion of the water used for irrigation, for example, is evaporated, transpired, and incorporated into plant biomass. This consumed water is unavailable for reuse in the watershed over time scales of practical interest. In contrast, after nonconsumptive use, water can be captured, treated, and reused. If a nonconsumptive use degrades the quality of the water (for example, by adding contaminants), it is said to generate wastewater. An example of nonconsumptive use is the flushing of a toilet, which converts drinking water into domestic wastewater. In principle, domestic wastewater can be collected, treated to remove human pathogens and other contaminants, and then reused for potable or nonpotable purposes. Globally, the largest consumptive use of water is for agriculture, whereas the largest nonconsumptive use of water is for industrial and municipal supplies (3).

What Is Water Productivity and How Can It Be Improved?

Addressing threats to human water security and biodiversity will require getting the most out of locally available water resources. But what does that mean in practice? One way to evaluate water use is to consider its “productivity,” defined as the value of goods and services produced per unit of water used. By improving water productivity, communities can enjoy the same goods and services, generate less wastewater, and leave more freshwater in streams, rivers, lakes, and coastal estuaries to support biodiversity. Because less water is harvested, treated, and transported, fossil fuel consumption and greenhouse gas emissions are reduced. Although water productivity has steadily improved in the United States since the mid-1970s, additional gains are possible both here and around the world (4). In this Review, we focus on three general strategies for improving water productivity (Fig. 1): substituting higher-quality water with lower-quality water where appropriate, regenerating higher-quality water from lower-quality water by treatment, and reducing the volume of higher-quality water used to generate goods and services.

What Are the Opportunities for Substituting?

Many municipal, industrial, and agricultural uses can be satisfied by lower-quality water. For example, treated domestic wastewater that would not be suitable for municipal water supplies may be perfectly suitable for industrial cooling and landscape irrigation, to name a few (5). Although the use of treated wastewater in the United States is currently limited (<5% of municipal supply), it could be expanded to 17 teraliters per year (Tl year⁻¹) (~27% of municipal supply), providing a new drought-resistant source of water in coastal areas where treated wastewater is currently discharged to the sea (6). Large-scale (centralized) wastewater treatment and potable substitution schemes can reduce overall energy consumption and reduce greenhouse gas emissions. In southern California, substituting potable water with treated wastewater consumes less energy and generates fewer greenhouse gases as compared to interbasin transfers of water or desalination of seawater or brackish groundwater (7).

Treated domestic wastewater is not the only lower-quality water that can be exploited in potable substitution schemes. Hong Kong’s dual water system, which has been in operation for over 50 years, supplies seawater for toilet flushing to 80% of its 7 million residents, cutting municipal water use in the city by 20% (8). A triple-water distribution system at Hong Kong’s International Airport, consisting of freshwater, seawater, and treated graywater from sinks and aircraft wash-down, cuts municipal water use by over 50% (8).

Potable substitution can also be implemented at neighborhood and single-home scales (Fig. 2). Rainwater (from roofs) and graywater (from

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laundry, dishwashing, and bathing) can be used in place of drinking water for a variety of activities. The reuse of graywater for toilet flushing and yard irrigation can cut household municipal water use by 50% or more (9). The energy cost, water savings, and reliability associated with rainwater harvesting depend on engineering considerations (e.g., contributing roof area and storage tank volume), local climate, connected end uses (e.g., toilet, laundry, and hot water), and temporal patterns (10). In a case study of a model home in Melbourne, Australia, the use of rainwater tanks to supply water for laundry, dishwashing, toilets, and an outside garden reduced household municipal water use by 40% (9). However, even in Melbourne, where rainwater-harvesting schemes are commonplace, they contribute a modest 5 gigaliters (GL) year⁻¹ to the city's overall water budget, which represents 1.2% of the city's total water use and 1.4% of its municipal supply (11).

Stormwater runoff from roads and other impermeable surfaces is another locally available source of water, but here the challenge is harvesting and storing the runoff (which can be generated over very short periods of time) and adequately removing contaminants (pathogens, metals, and organic pollutants). These challenges can be overcome through the integration of natural treatment systems into the urban landscape, including green roofs, rain gardens, bio-filters, and constructed wetlands (12). Processes responsible for pollutant removal in natural treatment systems include (12–15) gravitational sedimentation of large particles, pathogen removal by solar ultraviolet (UV) inactivation and predation, filtration of colloidal contaminants, oxidation of labile organics by hydrolysis and sunlight-generated reactive oxygen species, precipitation of metals, and nitrogen removal by bacterially mediated nitrification and denitrification in sediments. Plants play a key role, taking up excess nutrients and serving as both a source of organic carbon to fuel denitrification, and a source of oxygen through their root systems to fuel nitrification. As runoff moves through natural treatment systems, a portion of the water returns to the atmosphere (evapotranspiration); a portion infiltrates into the subsurface (groundwater recharge); and the rest can be harvested, stored, and ultimately used for nonpotable purposes. In Melbourne, stormwater harvesting is a relatively minor component (5 GL year⁻¹ or 1.4% of

municipal water use) of the city's water budget (11), but including stormwater reuse schemes in new greenfield and brownfield developments until 2050 could result in a sevenfold increase in nonpotable water availability for the city (35 GL year⁻¹ or 9.8% of municipal water use) (16).

Integrating natural treatment systems into urban landscapes confers many benefits beyond improving human water security. In warmer climates, the evapotranspiration of runoff moderates the urban heat island effect (17), whereas infiltration recharges the groundwater and provides environmental water for local wetlands and riparian zones (12). The construction of new

What Are the Opportunities for Regeneration?

With adequate treatment, higher-quality water can be regenerated from wastewater. Because additional goods and services are produced every time a parcel of water is recycled, regeneration has the potential to significantly increase water productivity. A prime example of regeneration is potable reuse, in which wastewater is treated with conventional and advanced methods and then added back to the water supply either directly (direct potable reuse) or indirectly, by holding the water for a time in groundwater or surface-water reservoirs (indirect potable reuse) (5, 6).

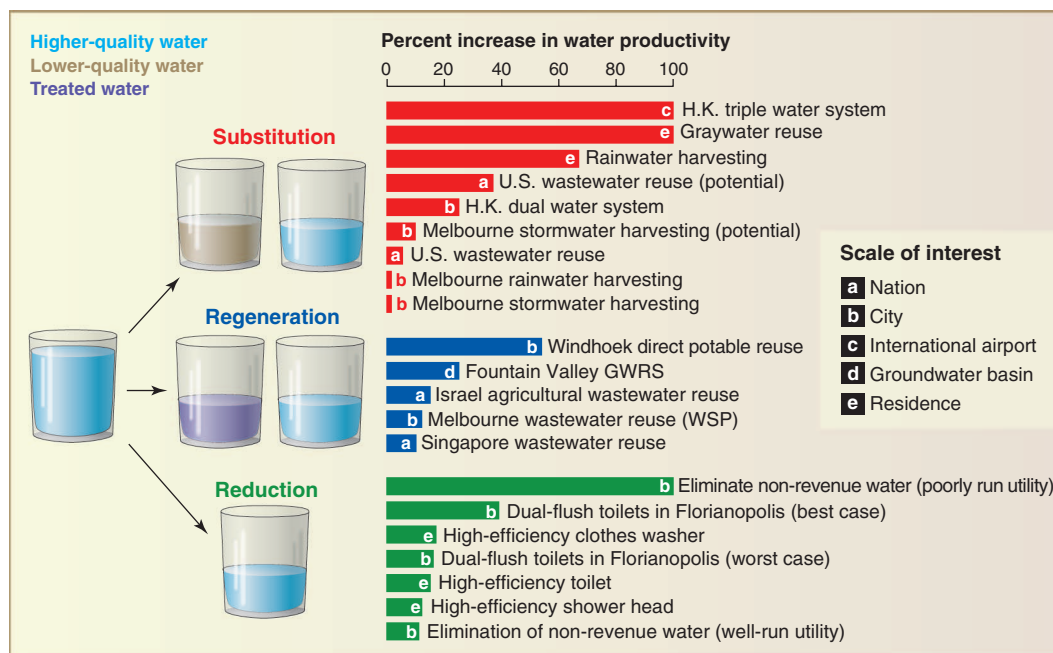


Fig. 1. (Left) Three complementary approaches for improving the productivity of higher-quality water. The water level in each glass shows how much water is used in producing a fixed value of goods and services. Substitution uses lower-quality water in place of higher-quality water for some activities. Regeneration transforms lower-quality water into higher-quality water by treatment. Reduction achieves the same value of goods and services using less higher-quality water. In these hypothetical examples, each option cuts by half the use of higher-quality water and therefore doubles its productivity. **(Right)** Percent increase in water productivity associated with the 21 case studies described in the text (51). These productivity improvements are illustrative only and will vary substantially in practice. The scale at which a particular water-saving intervention was implemented is indicated. The bars are color-coded to match the three general approaches for improving water productivity.

wetlands or reinvigoration of existing wetlands creates habitats for resident and migratory species and sustains biodiversity by enhancing habitat heterogeneity, connectivity, and food web support (18). When storm water is locally detained and retained throughout the catchment, less runoff enters rivers and streams, pollutant loads are reduced, and flow regimes more closely resemble predevelopment conditions (19). As a result, streams are less likely to overtop their banks and cause flooding (20), and the negative effects of urbanization on stream health and function, collectively known as the “urban stream syndrome” (21), can be mitigated (22).

Apart from a few small-scale facilities, direct potable reuse is not practiced in the United States. However, several indirect potable reuse facilities are operational. The world's largest is the Groundwater Replenishment System (GWRS) in Fountain Valley, California, which treats up to 97 GL year⁻¹ of domestic wastewater using conventional (primary and secondary sewage treatment) and advanced (microfiltration, reverse osmosis, and UV disinfection) techniques (23). Water produced by the GWRS provides approximately 20% of the water needed to maintain the local groundwater aquifer in Orange County, a primary source of municipal supply for more than 2 million residents.

Internationally, the longest-running example of direct potable reuse is in Windhoek, Namibia, where recycled wastewater (mostly domestic sewage) has been added to the potable water distribution system more or less continuously since the late 1960s with no obvious adverse health effects among the population of several hundred thousand (24). The current facility produces enough water (7.7 GJ year^{-1}) to meet approximately 35% of the city's municipal water needs.

Among the centralized options for augmenting potable water supplies, potable reuse is preferable to interbasin water transfers for several reasons (25): (i) Interbasin water transfers reduce the water available at the source for critical ecosystems and agricultural production; (ii) transporting water over long distances can be energy- and carbon-footprint-intensive; and (iii) the water transmission systems are vulnerable to disruption by natural and human-made disasters, such as earthquakes and acts of terrorism. All three problems are evident in California, where the southern part of the state has long relied on water imported from sources located hundreds of kilometers to the east and north. In 2001, an estimated 4% of

the electric power consumption in California was used for water supply and treatment (largely transportation) for urban and agricultural users; this estimate increases to 7% if end uses in agriculture (which are mainly related to pumping) are included (26). The depletion of source waters in the state has led to habitat deterioration, the decline and extinction of native fish species, the near-collapse of the Sacramento–San Joaquin River Delta ecosystem (27), and the desiccation of Owens Lake, whose dry lake bed is arguably the single largest source of asthma- and cancer-inducing respirable suspended particles in the United States (28). Potable reuse also has advantages relative to the desalination of seawater. By one estimate, potable reuse consumes less than one-half the energy [~ 1000 to 1500 kilowatt-hours per megaliter (kWh MI^{-1})] beyond conventional treatment required for the desalination of seawater (~ 3400 to 4000 kWh MI^{-1}) (25).

Relative to the classification scheme presented in Fig. 1, some nonpotable wastewater reuse is best described as regeneration, provided that the treated effluent replaces water of equal or lower quality, such as river diversions (Fig. 2). For exam-

ple, 73% of Israel's municipal sewage is treated and reused for agricultural irrigation, which is equal to roughly 5% of the country's total water use (29) and 13% of its municipal supply. In Singapore, 27 GJ year^{-1} of highly treated domestic wastewater is used primarily for industrial applications, which is equal to 5% of its total water use and 9% of its municipal supply (30).

Relatively low-energy centralized approaches for nonpotable wastewater reuse are also available, such as waste stabilization ponds (WSPs), in which sewage is directed through a series of open-air shallow ponds where physical processes (flocculation and gravitational sedimentation), microbial processes (algal growth, aerobic and anaerobic heterotrophic metabolism, nitrification, and denitrification), and exposure to sunlight jointly remove pathogens, organic contaminants, and nitrogen (31). Effluent from WSPs can irrigate crops (Fig. 2) or recharge groundwater aquifers, and the ponds themselves may provide a much needed quasi-wetland habitat for waterbird conservation (18). The world's largest WSP system, the Western Treatment Plant in Melbourne, produces 40 GJ year^{-1} of treated wastewater, equivalent to 11%

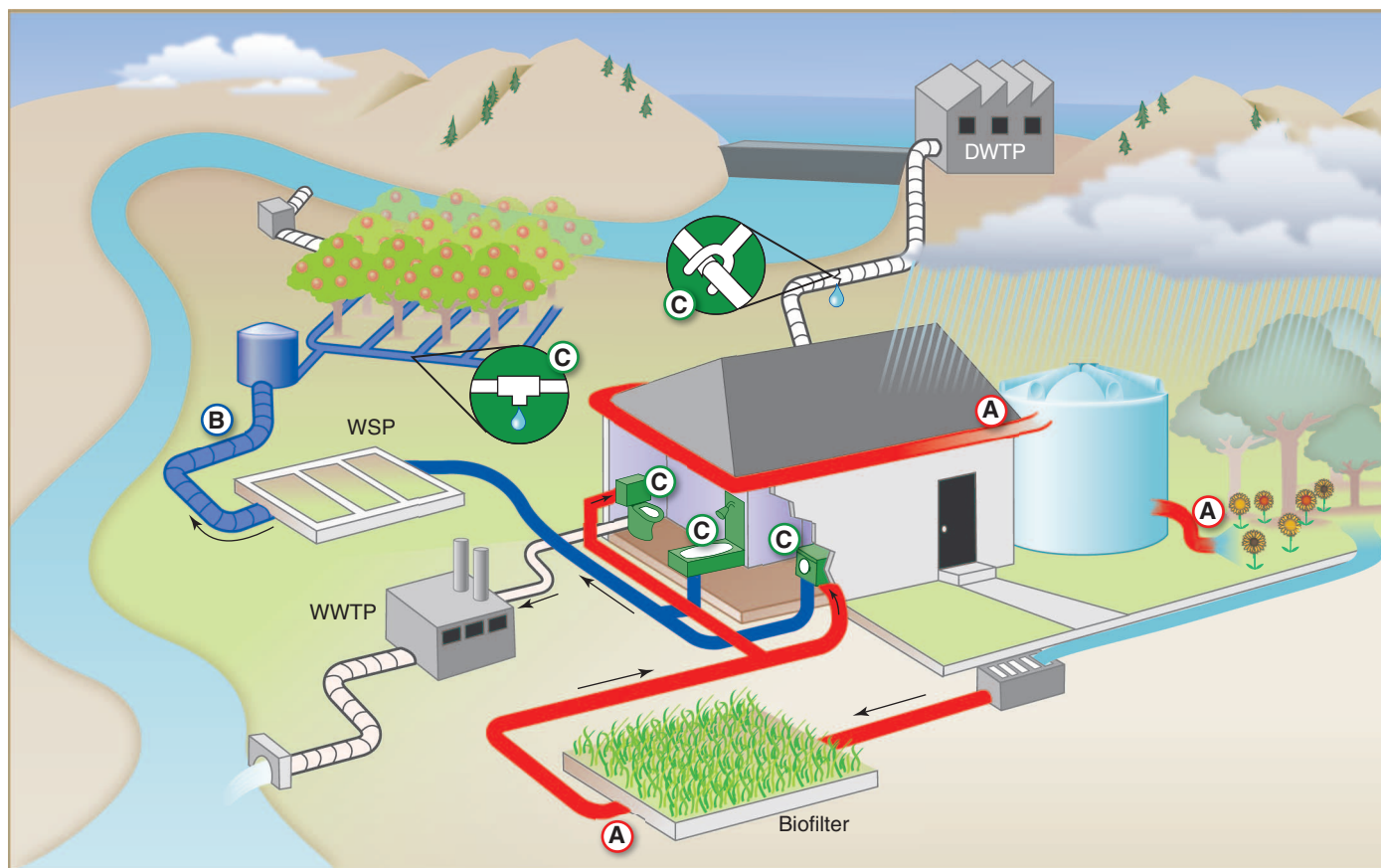


Fig. 2. Practical examples of substitution (A), regeneration (B), and reduction (C) at the household scale. Substitution includes watering a garden with rainwater from a rainwater tank and flushing toilets and washing laundry with treated stormwater effluent from a biofilter. For regeneration, a waste stabilization pond (WSP) transforms sewage from the house into high-quality water used for irri-

gating an orchard. Reduction includes repairing leaks in the water distribution system, drip irrigation, a dual-flush toilet, a low-flow shower rose, and a front-loading clothes washer. Other water infrastructure elements shown include a conventional drinking water plant (DWTP); a conventional wastewater treatment plant (WWTP); and a river diversion (supplying the orchard).

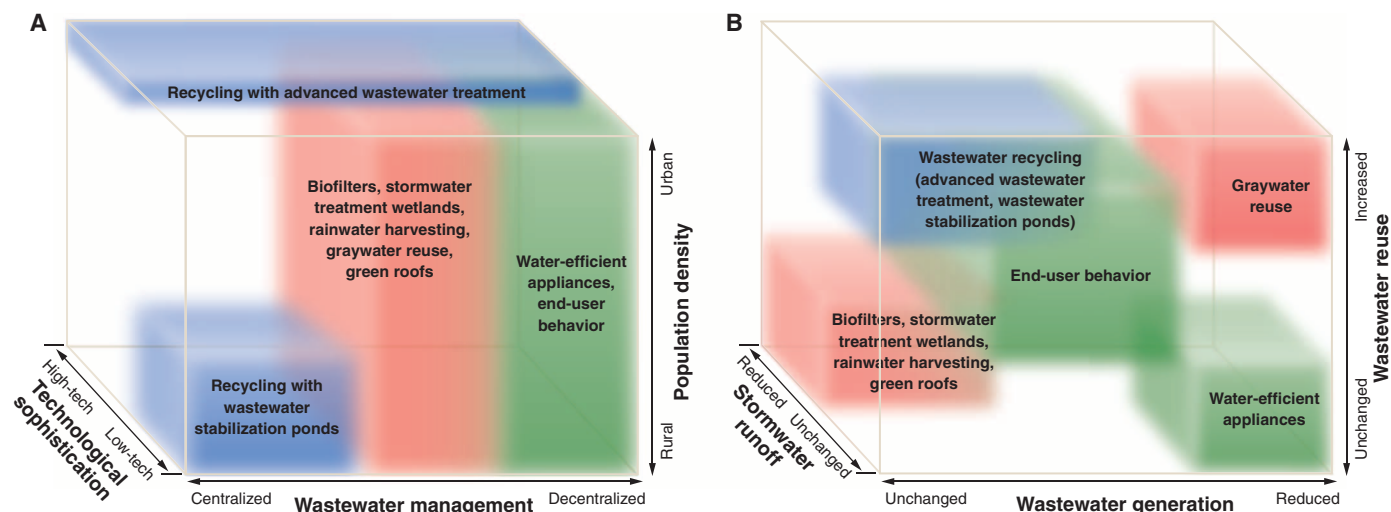


Fig. 3. Wastewater reuse and water-saving schemes discussed in the text, arrayed relative to a few key attributes (**A**) and performance indicators (**B**). The color scheme matches that in Fig. 1; substituting, red; regenerating, blue; and reducing, green. Certain schemes can be classified in more than one way;

for example, wastewater recycling with wastewater stabilization ponds might be considered blue or red, depending on the end use and what water is being substituted or regenerated. The adoption of water-saving schemes is influenced by policy, law, regulations, markets, and incentives.

of Melbourne's municipal supply, and uses approximately 500 kWh MI⁻¹ less energy than conventional wastewater treatment (32). Recycled water from the Western Treatment Plant is used for a variety of nonpotable applications, including in-plant uses and dual pipe schemes for the irrigation of agricultural crops, gardens, golf courses, and conservation areas.

Primary concerns associated with wastewater reuse include the buildup of contaminants and salts in soils (in the case of wastewater irrigation) and the possibility that incomplete removal of chemical or microbiological hazards during treatment may cause disease in an exposed population (6). Disease risk can be evaluated on a case-by-case basis using a statistical framework, such as quantitative microbial risk assessment, that predicts a population's disease burden, given the types and concentrations of pathogens that are likely to be present in the water, as well as particular exposure scenarios (33).

What Are the Opportunities for Reduction?

Water productivity can also be improved by reducing the volume of water used to produce a fixed value of goods and services. A modeling study of the water supply system in Florianopolis, Brazil, concluded that replacing single-flush toilets with dual-flush toilets would reduce municipal water use in the city by 14 to 28% and reduce energy use at upstream (drinking water) and downstream (wastewater) treatment plants by 4 GWh year⁻¹—enough energy to supply 1000 additional households (34). An analysis of 96 owner-occupied single-family homes in California, Washington, and Florida concluded that the installation of high-efficiency showerheads, toilets, and clothes washers reduced household use of municipal water by 10.9, 13.3, and 14.5%, re-

spectively (35). Because water is not technically required for bathroom waste disposal, the installation of composting toilets and waterless urinals can reduce municipal water use even further (36).

Agriculture accounts for the majority of global freshwater withdrawals (37), and thus even small improvements in water productivity in this sector can result in substantial water savings. Water savings can be achieved by switching to less-water-consuming crops, laser-leveling of fields, reducing nonproductive evaporation of water from soil or supply canals, changing irrigation scheduling, and adopting more efficient sprinkler systems, including microirrigation techniques (drip irrigation and microsprinklers) that precisely deliver water to plant roots (37). These approaches could help mitigate escalating water demand associated with growing energy crops, such as corn, particularly if projected increases in U.S. biofuel production are realized (38).

Drinking water is lost after it leaves treatment plants because of physical leaks in urban water distribution systems and poor accounting. Worldwide, the total volume of this "nonrevenue water" is estimated to be 49 Tl per year (39). Pipeline losses range from over 50% in much of the developing world to less than 10% in well-run utilities (39). The World Bank estimates that if just half of the losses in developing countries were eliminated, \$1.6 billion would be saved annually in production and pumping costs, and drinking water could be extended to an additional 90 million people without the need for new treatment facilities (39).

What Is the Role of Water Quality?

Protection of water quality is also a priority. The Catskill Mountains supply drinking water for New York City. When agriculture and residen-

tial developments threatened surface-water quality, the city considered building an \$8 billion water treatment plant, but instead opted to spend \$1 billion buying land and restoring habitat in the water supply catchment. This approach obviated the need for a treatment plant, saved the city billions of dollars in capital and ongoing operations and maintenance costs, and preserved a critical ecosystem (40).

What Is the Right Mix of Wastewater Reuse and Water-Saving Schemes?

The wastewater reuse and water-saving schemes described above are each tailored to a particular scale of implementation (from single homes to entire countries), population density (from urban to rural), and level of technological sophistication (from high-tech to low-tech) (Fig. 3A). Potable substitution schemes using advanced wastewater treatment may be feasible in an urban context, but not in a rural context. Furthermore, no single scheme simultaneously maximizes wastewater reuse, minimizes wastewater generation, and minimizes stormwater runoff (Fig. 3B). How does a community identify the right mix of schemes that will optimize their water systems? One study evaluated infrastructure options for a hypothetical residential development in the southeast of England, and concluded that every community has a technological state-of-the-art equilibrium beyond which tradeoffs are required (41). Wastewater reuse and water-saving schemes can improve water use, energy use, and land use up to the equilibrium point. Beyond the equilibrium point, further reductions in water use require increasing either energy use (if high-tech options are used) or land use (if low-tech options are used) (41). Human behavior should also be considered in the assessments of optimal water management strategies, as was

done in an elegant systems modeling study of water supply options in Chennai, India (42).

Because end-user behavior affects all aspects of water and wastewater management (Fig. 3B), changing attitudes and expectations may be more effective than finding infrastructure solutions to water scarcity. Turf grass consumes upward of 75% of residential drinking water in arid and semi-arid areas of the United States (43). If water resources in this region continue to dwindle, reducing the volume of water used for yard irrigation (for example, by implementing advanced irrigation technologies) may not be sufficient. Homeowners may have no choice but to replace turf grass with xeric landscaping (44). Such wholesale rethinking of our relationship with water is an example of a “soft-path” approach to water management. As a general principle, the soft-path approach is characterized by (4, 45) (i) viewing water as a service rather than an end in itself, (ii) adopting ecological sustainability as a fundamental criterion, (iii) matching the quality of water delivered to that needed by its use, (iv) planning from the future back to the present, and (v) ensuring community and citizen involvement in water management planning.

What Are the Main Roadblocks?

Efforts to improve water productivity will require overcoming economic, planning, regulatory, institutional, and public acceptance challenges. Key obstacles include uncertainty regarding the longevity and maintenance costs of infrastructure; upfront costs for piping, storage, and land; quantifying unpriced benefits; and overcoming water underpricing.

The first obstacle will probably resolve as experience is gained by vendors who develop wastewater-reuse and water-saving infrastructure, by community planning agencies that introduce them, and by municipal agencies or households that maintain them.

Underpricing of water is more serious because it leads to excess water demand and revenue shortfalls for water utilities. Underfunded utilities tend not to maintain infrastructure or repair leaks, adequately treat wastewater (spoiling scarce water resources), or extend service to the urban poor, who are then forced to buy expensive water from street vendors (46). Policies that inhibit full-cost pricing for water to ensure social equity or for other reasons may exacerbate this situation (47). When implemented in ways that ensure utility accountability to users and fair rate structures, full-cost pricing may help manage water resources sustainably and equitably (48), sending appropriate and realistic price signals that reflect the true cost (including externalities) of water use.

Many of the approaches discussed in this review require fundamental changes to the built environment, which can be promoted through a multi-objective approach that fosters collaboration with developers, a culture of innovation, and community

implementation capacity (49). Distributed water infrastructure can be introduced as part of comprehensive planning strategies that promote compact urban forms with mixed land uses and a focus on urban amenities, encourage alternative forms of transportation to permit narrower streets and reduce demand for parking, foster energy saving and waste recycling, promote water savings, and reduce liability for innovative developers.

Regulatory changes are needed to promote water conservation by mandating water-efficient plumbing, fixtures, and appliances. Regulatory changes are also needed to provide a consistent risk-management framework for water recycling schemes. Australian water reuse regulations, for example, emphasize protecting human health, which may foster a more favorable regulatory environment than in the United States, where water laws emphasize environmental health (6).

Finally, lessons from wastewater recycling systems indicate that the adoption of new water infrastructure depends on public acceptance. Public support for wastewater reuse, for example, is higher for uses such as landscape irrigation or car washing that minimize human contact (6). Public acceptance is also a necessary condition for utilities to embrace wastewater reuse and for firms to incorporate it in their production processes, provided that recycling wastewater neither substantially increases their costs nor triggers additional regulatory scrutiny.

To increase the likelihood of public acceptance, decision-makers should first demonstrate why changes are required to avert water shortages and that these water-saving schemes are safe. In addition, concerted and sustained efforts must focus on properly maintaining water infrastructure, especially when it pertains to wastewater reuse; on allowing stakeholders to monitor the uses and operations of wastewater recycling; and on vigilantly ensuring the protection of public and environmental health (50). Overcoming obstacles to the widespread adoption of wastewater recycling and water-saving measures is a *sine quo non* for meeting the water challenges of the future.

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 51. Water productivity P is defined as the value of goods and services V generated per unit of water used Q : $P = V/Q$.

The percent change in potable water productivity associated with the implementation of a water-saving scheme is $\Delta P = 100(P_2 - P_1)/P_1$, where P_1 and P_2 represent the productivity of potable water before and after the scheme is implemented (not all water-saving schemes discussed in the text directly reduced potable water use, but for consistency across the 21 case studies, water savings were benchmarked relative to potable water use at the appropriate scale). Assuming that the water-saving scheme does not change the value of goods and services produced, ΔP can be calculated

directly from potable (municipal) water use before (Q_1) and after (Q_2) scheme implementation:
 $\Delta P = 100(Q_1/Q_2 - 1)$.

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REVIEW

Conversion of Wastes into Bioelectricity and Chemicals by Using Microbial Electrochemical Technologies

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Waste biomass is a cheap and relatively abundant source of electrons for microbes capable of producing electrical current outside the cell. Rapidly developing microbial electrochemical technologies, such as microbial fuel cells, are part of a diverse platform of future sustainable energy and chemical production technologies. We review the key advances that will enable the use of exoelectrogenic microorganisms to generate biofuels, hydrogen gas, methane, and other valuable inorganic and organic chemicals. Moreover, we examine the key challenges for implementing these systems and compare them to similar renewable energy technologies. Although commercial development is already underway in several different applications, ranging from wastewater treatment to industrial chemical production, further research is needed regarding efficiency, scalability, system lifetimes, and reliability.

There is substantial energy in organic matter that is currently wasted or lost in treatment processes. Treatment of organic-rich wastewater currently consumes about 15 GW, or about 3% of all electrical power produced in the United States (1), but domestic, industrial, and animal wastewater together contain $\sim 1.5 \times 10^{11}$ kilowatt-hour (kWh) of potential energy (~ 17 GW of power) (2). Capturing part of this energy would provide a new source of electrical power that would also avoid the consumption of energy for wastewater treatment. Furthermore, agricultural practices could be modified to annually produce an additional 1.34 billion tons of biomass for energy production, without affecting food production (3), which is equivalent to more than 600 GW of continuous power. These different sources of waste organic matter can be a rich resource for energy production if we can develop cost-effective methods for harnessing this energy. Alternatively, we could capture this waste biomass energy in industrial pro-

cesses to make other useful chemicals, such as biofuels or industrial chemicals, that currently require electricity or organic substrates for this purpose.

Recently developed microbial electrochemical technologies (METs) that use microorganisms to catalyze different electrochemical reactions, such as microbial fuel cells (MFCs) that generated electrical power, are promising approaches for capturing the energy in waste biomass for diverse purposes. Energy production by electrochemical processes or conventional combustion requires a fuel to provide electrons and an electron acceptor (oxidizer). In METs, organic matter is the fuel, and oxygen is the primary oxidizer for aerobic respiration by bacteria. However, many other soluble chemical species can serve as oxidizers for anaerobic bacteria, including nitrate, sulfate, and carbon dioxide. Bacteria known as exoelectrogens have the ability to transfer electrons outside the cell to insoluble electron acceptors, such as iron and other metal oxides, or to electrodes in bioelectrochemical systems. The most commonly studied microorganisms are various *Geobacter* and *Shewanella* spp., but many other bacteria have been found to possess exoelectrogenic abilities (4). Electrons are transferred by these bacteria outside the cell indirectly, by using electron shuttles such as flavins and phenazines (5–7), or directly by using outer membrane proteins (8). These mechanisms can occur in combination with self-produced conductive pili called nanowires (9, 10). In con-

trast, electrotrophic microorganisms can directly or indirectly accept electrons into the cell (11).

How Do Microorganisms Generate Electricity from Organic Matter?

The use of exoelectrogenic microorganisms in MFCs allows electrical power generation from nearly any source of biodegradable organic or inorganic matter in water that does not directly require oxygen as a part of the degradation process. These organic sources include simple molecules such as acetate, ethanol, glucose, and hydrogen gas; polymers such as polysaccharides, proteins, and cellulose; and many types of wastewaters from domestic, food processing, and animal sources (12, 13) (Fig. 1). In an MFC, bacteria release electrons to the anode and protons into solution, resulting in a negative anode potential of about -0.2 V (versus a standard hydrogen electrode) that is generally only slightly more positive than that of the half-cell reaction for the substrate (e.g., a midpoint potential at pH = 7 of -0.28 V for acetate) (14). In most cases, oxygen in air is used as a sustainable oxidizer at the cathode, with a typical maximum potential of $+0.3$ V, producing an overall maximum cell potential of $+0.5$ V. Cathode potentials obtained in MFCs are considerably lower than theoretical values ($\sim +0.8$ V, with oxygen) even with Pt-catalyzed cathodes (15, 16) (Fig. 1). One of the most promising nonprecious metal materials used for oxygen reduction in MFCs is activated carbon, because it is both inexpensive and renewably produced from waste biomass (17). Nitrate is an alternate electron acceptor that produces comparable cell voltages because of its high solubility relative to oxygen (18). Voltages cannot be increased by linking MFCs in series as is done with batteries (19, 20). However, higher voltages can be captured from arrays of MFCs by wiring them to charge capacitors in parallel and then discharging the capacitors in series, resulting in nearly additive voltages from the individual MFCs (21).

The power densities produced by MFCs are lower than those possible by using hydrogen fuel cells because of high internal resistances, the limited temperature and solution conditions tolerated by microorganisms, substrate degradability, and biofilm kinetics. Hydrogen fuel cells use an ion-exchange membrane as a solid electrolyte for charge transfer. Membranes are not required in MFCs, and using a membrane between the anode and cathode can add internal resistance, which will

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reduce power (15). Instead of using ion-conductive membranes, many MFCs are being designed with separators or coatings on the cathode to insulate the cathode and protect it from biofilm growth (22). Water serves as the electrolyte for charge transfer in an MFC, with power increased in proportion to solution conductivity up to limits tolerated by bacteria. Charge transfer is different from a hydrogen fuel cell because charge is balanced primarily by ions more abundant in the water than protons, such as sodium and potassium. This results in pH gradients that can reduce performance (23, 24), although in some instances pH changes can be useful, as discussed below. Typical maximum power densities in MFCs are ~ 2 to 3 W m^{-2} of projected electrode (usually the cathode), under optimum conditions of $\sim 30^\circ\text{C}$, solution conductivities of $\sim 20 \text{ mS cm}^{-1}$, and well-buffered solutions at neutral or slightly alkaline pH. MFC power densities are much lower than those in hydrogen fuel cells, which produce on the order of 1 W cm^{-2} , but these power densities are consistent with chemical fluxes into biofilms based on an analogous flux in terms of equivalent electrons (for example, 24 mol e^- per mol glucose) (14). Upper limits of 17 to 19 W m^{-2} have been predicted for MFCs on the basis of estimates assuming minimal internal resistance or by assuming first-order kinetics typical of microorganisms in biofilms (4, 25). Increasing electrode-packing

densities (electrode area per volume of reactor) has produced up to 1.55 kW per m^3 of reactor volume (2.77 W m^{-2}) under optimum conditions (26), which is much higher than typically produced from wastewaters ($<0.5 \text{ W m}^{-2}$) (27, 28). Lower power densities obtained with wastewaters are due to slower biodegradation kinetics for complex substrates, lower solution conductivities, and reduced buffer capacities.

The complex nature of many waste biomass sources requires a diverse microbial community to degrade the various components of the organic matter. Although many bacteria may produce electrical current, high power densities from complex sources of organic matter are typically associated with the presence of *Geobacteraceae* in the anodic community (4, 29). These microbes use a limited number of substrates, with acetate being the most common among different strains, although lactate and a few others are also possible substrates (30, 31). Syntrophic interactions can therefore be required to break down complex organic matter into simpler substrates that can be used by exoelectrogens (Fig. 1) (29). The nature of the syntrophic associations is highly similar to anaerobic digestion, where diverse substrates are broken down to simple molecules that are used for methane generation by members of the *Methanosarcinales* genus (32). Community asso-

ciations are not yet well understood in MFCs, with certain microbes affecting power disproportionate to individual contributions (33). For example, power was increased by 30 to 70% by adding Gram-positive *Enterococcus faecium* to cultures of Gram-negative *Pseudomonas aeruginosa*, even though pure cultures of *E. faecium* produced little power (34). Further studies demonstrated that *Enterococcus aerogenes* could produce 2,3-butanediol, leading to increased production of an electron shuttle (phenazine) by *P. aeruginosa* (35).

What Types of Harvestable Chemicals Can Microorganisms Produce in Bioelectrochemical Systems?

Applying electrical power to a bioelectrochemical system can enable the generation of many different chemical products from biomass, such as biofuels (36). This modification is commonly referred to as a microbial electrolysis cell (MEC), based on the first systems that produced hydrogen gas through the electrolysis of biomass by bacteria as opposed to water electrolysis. Energy is added into an MEC by either using an external power source or setting an electrode potential using potentiostat. Hydrogen gas can be produced at the cathode at a theoretical potential of -0.41 V under standard conditions and neutral pH (36). Acetate oxidation by microorganisms could achieve an anode potential down to -0.28 V , so a minimum of -0.13 V theoretically needs to be added into the system. This voltage is much less than that needed to split water (-1.2 V) because of the favorable thermodynamics of organic matter degradation compared with the energy-demanding reaction for splitting water. Typically more negative voltages than -0.3 V are used, even with Pt catalysts, to increase gas production rates. Applied voltages down to about -1 V allow more energy to be captured in the hydrogen gas produced than the electrical energy used. However, energy efficiency is only part of the cost of producing hydrogen gas, and higher voltages may be used to make gas production cost effective.

Microorganisms can be used on the cathode to directly (via electron transfer) or indirectly (through evolved chemicals) catalyze the production of inorganic chemicals. Electrotrophic microbes are not true catalysts because they derive energy from this process. The first biocathodes used to produce methane required mediators (37) or hydrogen gas (38), but later it was shown that methanogenic communities could directly accept electrons from the cathode (39). Methane is commonly produced in MECs, primarily from hydrogen gas evolved from the cathode but also through acetoclastic methanogenesis (38, 40). Although only a small amount of H_2 gas is typically lost in small single-chamber laboratory MECs (41), methanogenesis can eliminate hydrogen gas recovery in larger systems (42, 43) unless the gas produced at the cathode is kept separate from methanogens (44). Hydrogen gas can be produced using biocathodes (45), although the energy efficiencies are lower

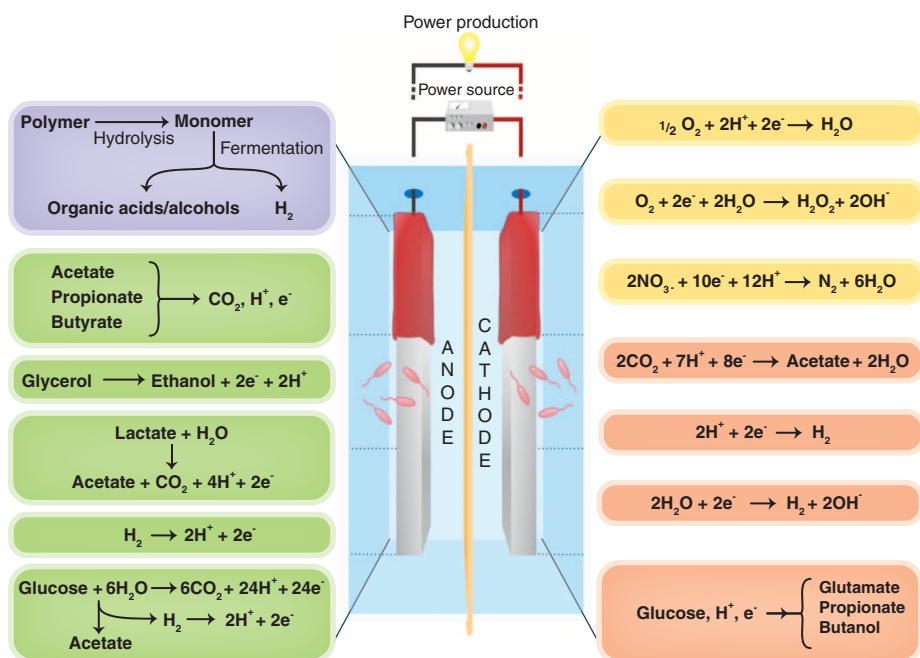


Fig. 1. Overview of anodic and cathodic reactions in a bioelectrochemical system. Electrode reactions are due to planktonic or biofilm cells or in some cases are directly electrochemically catalyzed. At the anode, polymeric material can be degraded to simple molecules, such as fatty acids and hydrogen, which can be used for current generation. Other bioconversion reactions, such as conversion of glycerol to ethanol, can also produce compounds for current generation. At the cathode, reactions can result in power generation or additional product formation. Purple indicates reactions that do not directly result in current generation; green, reactions that can produce current; yellow, reactions that can occur spontaneously or can be accelerated by adding additional power; orange, power addition is required. The stoichiometry of the reactions is principally theoretical because many conversions lead to side products as well as biomass formation.

than those of inorganic catalysts. Although a kilogram of methane has lower energy and economic value than the same mass of H_2 , the complete conversion of H_2 gas evolved in an MEC to methane can still produce a positive energy balance (43). The gas is also enriched in methane (86%) compared with an anaerobic digester because of the consumption of carbon dioxide.

The cathodic production of hydrogen peroxide or caustic solutions appears to be a promising application of MECs (Fig. 2). When a cation-exchange membrane is placed between the anode and cathode, pH gradients rapidly develop in the two chambers, even with mildly buffered solutions (23). This situation can be exploited to produce NaOH concentrations as high as $\sim 1 \text{ mol l}^{-1}$ in the cathode (46). Production of the caustic in an industrial setting avoids purchasing a high-cost concentrated solution and the need to transport these solutions to the site. Oxygen reduction at the cathode normally produces water, but, in the absence of an efficient catalyst such as Pt, the reaction results in a two-electron transfer and the production of hydrogen peroxide (47). An alkaline solution of hydrogen peroxide is a strong oxidant that can be used industrially for bleaching. A life-cycle analysis suggests that production of hydrogen peroxide in MECs is more sustainable than presently used routes (48).

MECs can also produce a variety of organic chemicals by electroautotrophic processes using CO_2 or from substrate organics via microbial electrosynthesis (MES) (49). Acetate and oxo-butyrate were produced by using a culture of *Sporomusa ovata* attached to a cathode that was the sole electron donor (50). Although multiple homoacetogenic cultures have now shown the ability to catalyze CO_2 reduction by using electricity as donor (51), thus far the rates and titers produced are very low. Major improvements in production rates will be essential to bring about microbial electrosynthesis based on CO_2 for large-scale applications. For several decades, it has been known that providing reducing power to fermenting cultures with use of electrochemical processes can redirect glucose fermentations toward glutamate, butanol, and propionate (52–54). The known exoelectrogen, *Shewanella oneidensis* MR-1, was engineered to oxidize glycerol to ethanol by discharging excess reducing equivalents to the anode (55). Acetate was converted to ethanol by using a microbial population in an MEC (56). Advantages of starting from waste organics are the lower electron requirement relative to CO_2 as well as the possibility to upgrade or treat a waste stream.

How Do METs Measure Up Against Conventional Technologies for Wastewater Treatment?

Current wastewater treatment processes have their bases in processes developed over a hundred years ago, and, although there have been incremental advances, new paradigm-changing approaches are needed to enable substantial energy recovery. Treatment of domestic wastewater in a conventional activated sludge process requires -0.3 kWh m^{-3} for aeration, with about twice that used overall for pumping and in other processes (1). Membrane bioreactors that can achieve efficient treatment and excellent water quality are being used more frequently, but they have high energy demands (-1 to -2 kWh m^{-3}). Some wastewater treatment

direct generation of electricity in METs can be similar to conventional fuel cells and lead to very high energy efficiencies because they do not have Carnot cycle limitations inherent in combustion processes. However, energy recovery in METs to date have been low, leading some to question whether they can ever compete with conventional processes (1). The maximum power densities produced in MFCs using domestic wastewater alone (without other energy sources) have reached 12 W m^{-2} (27), equivalent to 0.07 kWh m^{-3} produced over 6 hours (comparable to activated sludge treatment times). This energy recovery is low considering that domestic wastewater contains $\sim 2 \text{ kWh m}^{-3}$ (58).

A more feasible role for wastewater is as an energy source for chemical production in METs, with the bulk of the treatment accomplished by using more conventional processes. By producing chemicals such as H_2 and hydrogen peroxide from wastewater instead of through electrolysis or other means, the energy contained in the organic matter is used to supply at least part of the production energy. As shown by Foley *et al.* (48) in a life-cycle analysis comparing anaerobic digestion, MFCs, and MECs, on-site production of chemicals can bring about a considerable environmental benefit to the overall treatment process. It is therefore important to consider whether the primary goal of using wastewater in METs is complete treatment or instead the production of useful chemicals as a part of treatment.

Combining METs with Other Emerging Renewable Energy Technologies

Reverse electrodialysis (RED) is a process that can be used to directly create electrical power from a salinity gradient (59). The flow of seawater and freshwater (or treated wastewater) through pairs of ion-exchange membranes in a RED stack creates an electrical potential of 0.1 to 0.2 V per pair of membranes. Globally, up to 980 GW of power could be harvested from salinity gradient energy where freshwater flows into the sea (60). A RED stack can be placed between the anode and cathode chambers of an MFC or MEC, creating a hybrid technology called a microbial reverse electrodialysis cell (MRC). The favorable anode reaction provided by exoelectrogenic bacteria degrading organic matter allows MRCs with oxygen reduction at the cathode to produce three times the voltage (1.2 V) and 6.1 times the power (4.3 W m^{-2}) of an MFC lacking the membrane stack with NaCl solutions (61). The use of the RED stack in an MEC produced H_2 gas at energy efficiency of

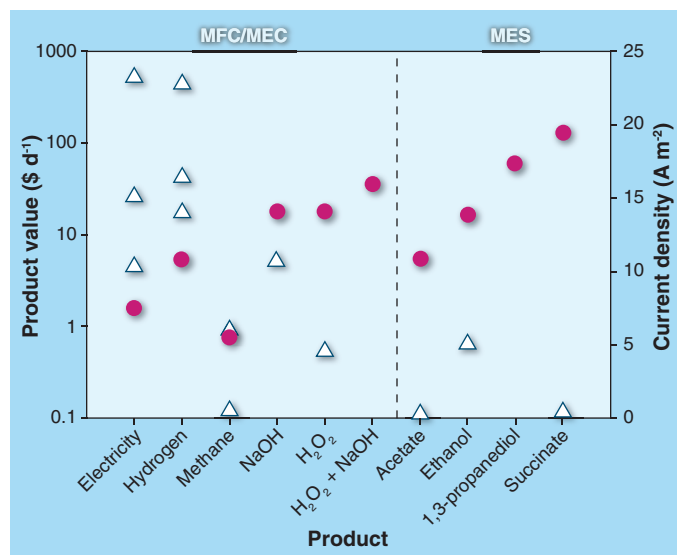


Fig. 2. Possible outcomes using different microbial electrochemical technologies in terms of economic values (calculated for 1 day at 1000 A and neglecting the cost of input materials) and current densities (exemplary for state of the art). There is roughly an inverse relationship between the value of these products (triangles) and the current densities (circles), which is in part due to the more effort in producing the lower-value products such as electricity and biofuel gases (hydrogen and methane). Generating 1000 A translates to a theoretical consumption of 7.16 kg waste organic matter (expressed as chemical oxygen demand).

plants using activated sludge bioreactors have become energy neutral through the combination of nutrient removal with anaerobic digestion of sludge that produces biogas (methane) (57). Principal limitations of anaerobic digestion as the main treatment process (i.e., replacing activated sludge) are the need for a concentrated waste stream ($>3 \text{ kg}$ organic matter per m^3) and warmer temperatures ($>20^\circ\text{C}$), and the process is only economical by using very large digesters. For these reasons, anaerobic digesters have only been used in conventional treatment plants for treatment of the sludge and not the main flow.

The strength of METs is their applicability to directly treat the wastewater while substantially decreasing the need for sludge handling and treatment resulting from low solids production. In theory, the

up to 65%, without the need for electrical grid energy (62). Low-grade waste heat can be used to generate salinity gradients (high and low concentrations of salt solutions) from thermolytic chemicals such as ammonium bicarbonate (63). MRCs using ammonium bicarbonate have produced 3 W m^{-2} (4.5 a.m.^{-2}) with domestic wastewater and 5.6 W m^{-2} with acetate (30% energy efficiency, not including distillation energy) (64). Thermolytic solutions enable the use of MRCs at any location where there is wastewater or waste biomass and waste heat. The use of thermolytic solutions also minimizes biofouling of the membranes, which is problematic when using seawater and river water.

What Are the Prospects for Scaling Up and Commercialization?

MFCs and MECs are exciting but nascent technologies, with many opportunities and challenges for successful applications. One key factor for commercial success of these technologies will likely be lowering the cost of electrodes and associated materials to enable recovery of capital costs within only a few years. The electrodes need to have high surface areas and be tightly packed together to maximize power volumetric densities, while at the same time allowing a complex interaction of microorganisms with the wastewater (Fig. 3). Rising costs for producing electrical power coupled with incentives for carbon-neutral processes could bring these systems into practice, with sufficient investment, in the near future.

Several companies are now in the process of commercializing MFCs and MECs for various applications, including wastewater treatment and the production of biochemicals, caustic solutions, and hydrogen peroxide solutions. The main challenge of using MFCs as a stand-alone method for electrical power generation is the low value of electricity. For the same reason, it may be difficult to realize a profit on investment by using MFCs as a stand-alone method of wastewater treatment because of little profit that can be made from producing electricity. The primary economic benefit for wastewater treatment is reducing, or completely avoiding, the need for electrical power consumption for aeration. METs offer many more opportunities for novel applications than other processes such as anaerobic digestion, which do not require wastewater aeration, because they can be used to produce many different products. Although biochemical production offers additional economic incentives, the need for high current densities when making a product could preclude result in effluent organic matter concentrations that are above those allowable for direct discharge. The integration of METs into domestic and industrial sites will therefore require careful consideration of the specific opportunities at different sites and the costs involved in partial or full treatment with this bioelectrochemical approach. The overall benefits to be realized from METs provide great incentives for continued innovations,

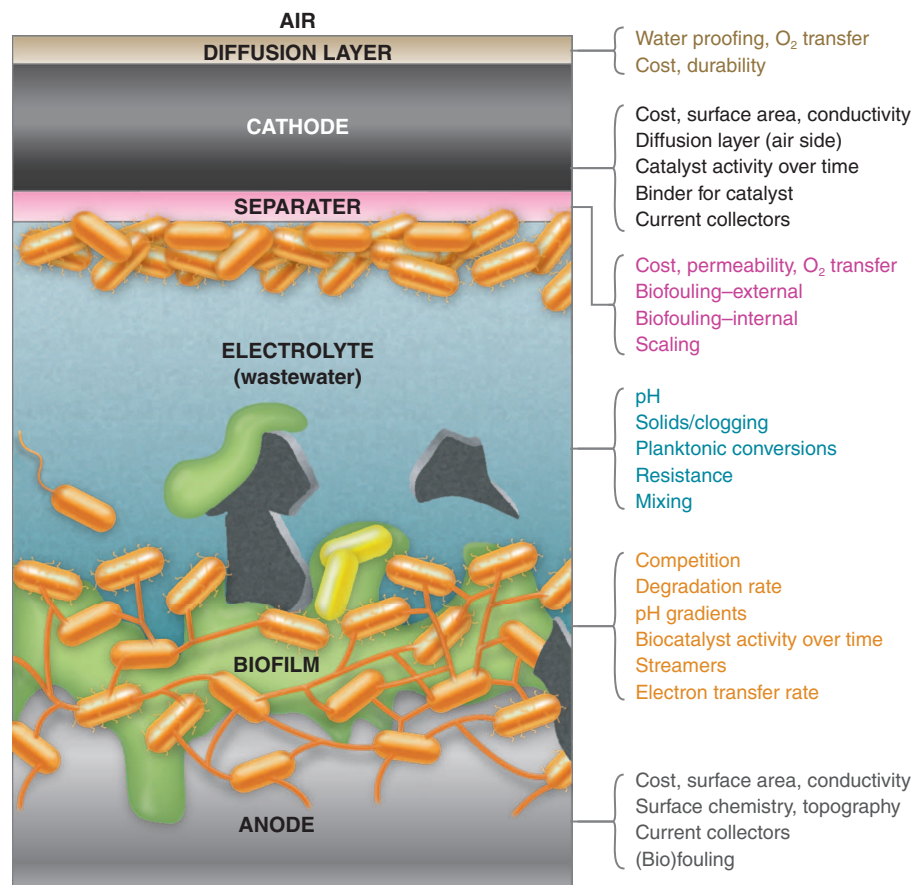


Fig. 3. The complex environment of the MFC presents many opportunities for scientific study as well as challenges for commercial applications. An electrogenic biofilm colonizes the anode, and its development depends on the specific electrode materials and properties. Within the biofilm, inert material (solids or dead cells, dark gray) can limit access for the cells to the electrode. Electroactive cells (orange) can be in direct or indirect contact with the electrode; competing cells (yellow, e.g., methanogens) do not require this contact and can thus have a competitive advantage outside of the biofilm. pH gradients can build up in the biofilm and bulk solution (electrolyte), leading to decreased performance and loss of electrochemical potential. Solids in the wastewater can lead to clogging, and planktonic cells may show competitive behavior for organic matter with the biofilm. Protons (anode) or hydroxyl ions (cathode) generated at the electrodes need to be transported to the counterelectrode, and competition with other more-abundant ions (such as sodium and chloride) can lead to changes in the bulk pH, which in turn affects the process. The cathode will catalyze oxygen reduction, but it can foul with bacteria and organic and inorganic matter, leading to reduction in catalysis. Oxygen can leak through the cathode and separator, resulting in loss of organic matter and decreased power.

developments, and applications such as energy and chemical production from waste biomass.

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REVIEW

Challenges in Metal Recycling

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Metals are infinitely recyclable in principle, but in practice, recycling is often inefficient or essentially nonexistent because of limits imposed by social behavior, product design, recycling technologies, and the thermodynamics of separation. We review these topics, distinguishing among common, specialty, and precious metals. The most beneficial actions that could improve recycling rates are increased collection rates of discarded products, improved design for recycling, and the enhanced deployment of modern recycling methodology. As a global society, we are currently far away from a closed-loop material system. Much improvement is possible, but limitations of many kinds—not all of them technological—will preclude complete closure of the materials cycle.

The generation now between the ages of 20 and 30 is, in many parts of the world, the first to have grown up with the recycling bin as a normal part of life. Discarded paper, cans, and bottles have designated places to go, and often go there. The situation is less certain for products used for a number of years before being discarded—computers, refrigerators, automobiles—for which recycling procedures have been diverse and sporadic. And few know what happens to obsolete equipment used on behalf of individuals but owned by corporations or organizations—medical imaging machines, aircraft engines, and the like.

The recycling of products in the “occasionally discarded” or “owned by somebody else” categories is complicated by the rapid expansion of

the designer’s materials palette that has taken place in the past several decades (1, 2). Today, virtually every stable element in the periodic table is used so as to take advantage of its unique physical and chemical properties. The result is that many products are more functional and reliable than before. An unintended consequence is that recycling has become much more complicated and challenging.

Several reviews of metal recycling have appeared in recent years (3–5). They discuss central issues such as recycling technologies, economic limitations, and methods of enhancement. Some open questions still remain: How much is going on, and what are the trends? What are its limits? Is a closed-loop materials economy possible? It is these systems-level topics that are the focus of the present work.

The Current Status of Metal Recycling

How well is the world doing at recycling the diverse mix of elements in modern products? Two

metrics answer this question best: recycled content and end-of-life recycling rate (EOL-RR). Recycled content describes the share of scrap in metal production, which is important to get a sense of the magnitude of secondary supply. This indicator, however, has two limitations. First, lifetimes of metal-containing products often span several decades, which, in combination with rapid growth in metal use, means that recycled metal flows will meet only a modest portion of demand for many years to come. Second, it does not distinguish between new (yield loss from fabrication and manufacturing) and old (postconsumer) scrap as input material, making it vulnerable to artificially increased rates based solely on preconsumer sources (fabricators may be given incentives to increase their scrap output to meet secondary demand, making recycled content an incentive for inefficiencies in fabrication and manufacturing). What recycled content means to encourage, instead, is the amount of old scrap that is collected and processed for recycling [also expressed as old scrap ratio (6)]. The indicator that measures this more directly is the EOL-RR, defined as the fraction of metal in discarded products that is reused in such a way as to retain its functional properties.

The EOL-RR depends on the collection rate of end-of-life products and the efficiency of the subsequent separation and pre-processing steps, all involving complex interactions of a wide variety of players (7). A United Nations panel recently defined and quantified recycling rates for 60 elements (Fig. 1) (8). Two messages jump out at once from the figure. The first is that EOL-RRs for the commonly used “base metals” (iron, copper, zinc, etc.) are above 50% (although, as the report is careful to point out, usually not very far above 50%). The second, and striking, impression

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is the number of elements that are seldom if ever recycled. It turns out that most of these are increasingly used in small amounts for very precise technological purposes, such as red phosphors, high-strength magnets, thin-film solar cells, and computer chips. In those applications, often involving highly comingled “specialty metals,” recovery can be so technologically and economically challenging that the attempt is seldom made. Overall, modern technology has produced a conundrum: The more intricate the product and the more diverse the materials set it uses, the better it is likely to perform, but the more difficult it is to recycle so as to preserve the resources that were essential to making it work in the first place.

The benefits of recycling are many, the most obvious being the potential to reduce the extraction of virgin ores, thus extending the life of those resources. The environmental impacts of metal production are reduced substantially when recycled materials rather than primary materials are used (9), and recycling a metal is generally much more energy-efficient than acquiring it from a mine (10–13). Depending on the metal and the form of scrap, recycling can save as much as a factor of 10 or 20 in energy consumption (14).

Factors influencing the recycling efficiency are the volumes involved and the economic value of the metal. Metals that are typically used in large quantities (enabling economies of scale) represent the largest fraction of currently recycled metals. These metals, which occur in relatively pure form and are straightforward to remelt, include steel, aluminum, copper, zinc, lead, and nickel. Their EOL-RRs are above 50%, and the lifetimes of the products in which they are used often span several decades. Recycling infrastructures are well established.

At the other end of the spectrum are metals used in only small amounts. “Specialty” metals are used to enable enhanced performance in modern high-technology products such as jet engines, solar cells, and consumer electronics. In such applications, mixing of materials is extensive, separation technology is challenging, and the economics are often unfavorable because of the small amounts involved. The trend to use specialty metals is increasing, and given the short lifetimes of many electronic devices, end-of-life losses will also increase sharply soon unless better recycling management options are found. Most of the materials shown in red in Fig. 1 fall into the specialty metals group [e.g., indium (15), rare earth elements (16)].

A special case of metals used in small amounts are those with high economic value, such as precious metals. Their value is a key incentive for

recycling (17), yet their end-of-life recycling rate is at best on the order of 60% (6). The reason is that despite high recycling rates for traditional uses in jewelry or industrial catalysts, the collection and recycling of platinum, palladium, and rhodium from automotive catalysts is more challenging. Here, collection rates fluctuate around just 50% in developed countries, largely as the result of exports of used vehicles to developing countries with minimal recycling technology (18). The same factors are also involved in the meager 5 to 10% recycling of platinum group metals in electronics (19). Within developing countries, informal recycling and low-technology processing combine to sharply limit the recovery of precious metals from consumer products (20). Hazardous metals recycling takes place only

flue gas, the disposal problem is only shifted to subsequent processing steps such as landfills, as the incineration process does not change the stable structure and properties of these materials (26), which is likely also the case in recycling processes.

Metal life cycles from cradle to grave. The potential for recycling depends on approaches and actions taken at each stage of the life cycle. This can be illustrated by example (Fig. 2A). The left panel shows the 2005 global life cycle for nickel (27). Of the 650 Gg (thousands of metric tons) of nickel that were discarded from use, about two-thirds was returned. Together with manufacturing scrap (165 Gg of Ni), recycled nickel provides about one-third of the nickel required for fabrication and manufacturing—obviously well worth doing, but with the potential for further im-

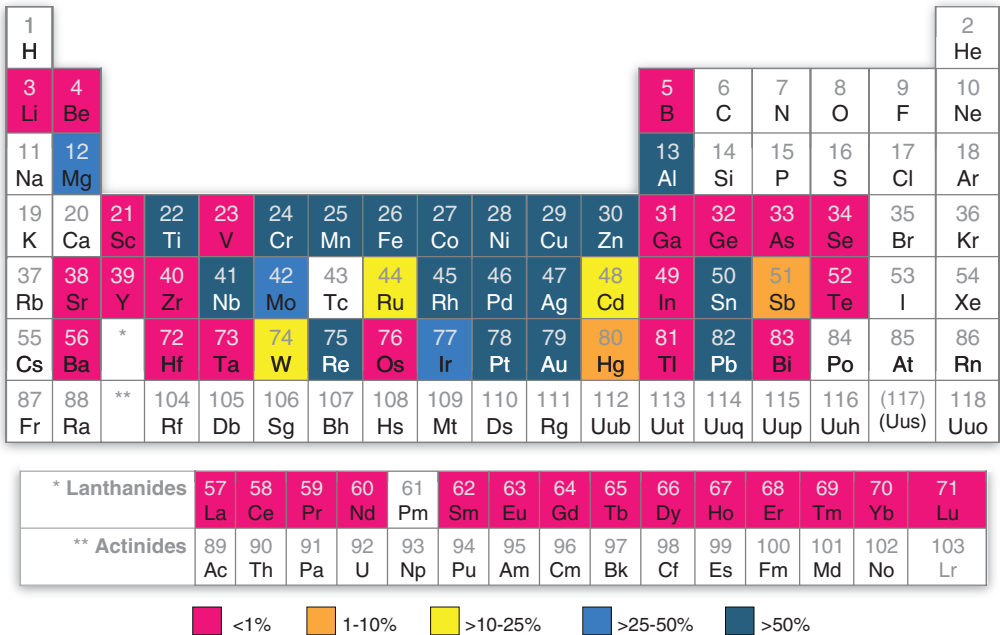


Fig. 1. Global estimates of end-of-life recycling rates for 60 metals and metalloids, circa 2008 [adapted from (6)].

occasionally and at low rates. Cadmium is mostly recycled in the form of nickel-cadmium batteries, where low collection rates limit the recycling efficiency (21, 22), and global recycling rates of mercury-containing fluorescent light bulbs are found to range at best from 10 to 20% (23). Lead is an exception. Eighty percent of today’s lead use is for batteries (24, 25) in gasoline- and diesel-driven automobiles and for backup power supplies, and collection and pre-processing rates from these uses are estimated to be within 90 to 95% as a result of stringent regulation worldwide (25). The result is a nearly closed-loop system for lead use in batteries.

Ecotoxicity challenges can also arise from the disposal of metal-containing nanomaterials. Although modern solid-waste incinerators are found to efficiently remove engineered nanomaterials from

provement. By contrast, the right panel shows the 2007 global life cycle for neodymium (16). In this cycle, 15.6 Gg of Nd was used in fabrication and manufacturing, but only 1.2 Gg of Nd was discarded from use (mostly because products containing neodymium are rather recent arrivals on the market and have not yet become obsolete). Little to none of that material is currently being recycled, and if it were, it would not play a major supply role. In years to come and as discards mount, however, neodymium recycling has the potential to be of benefit. Although the two elements represent the two extremes in end-of-life recycling, it is sobering to note that even the overall life cycle efficiency of the more efficient one, nickel, is only 52%—that is, almost half of the extracted nickel is only used once before being lost as production waste, waste in landfills, or for

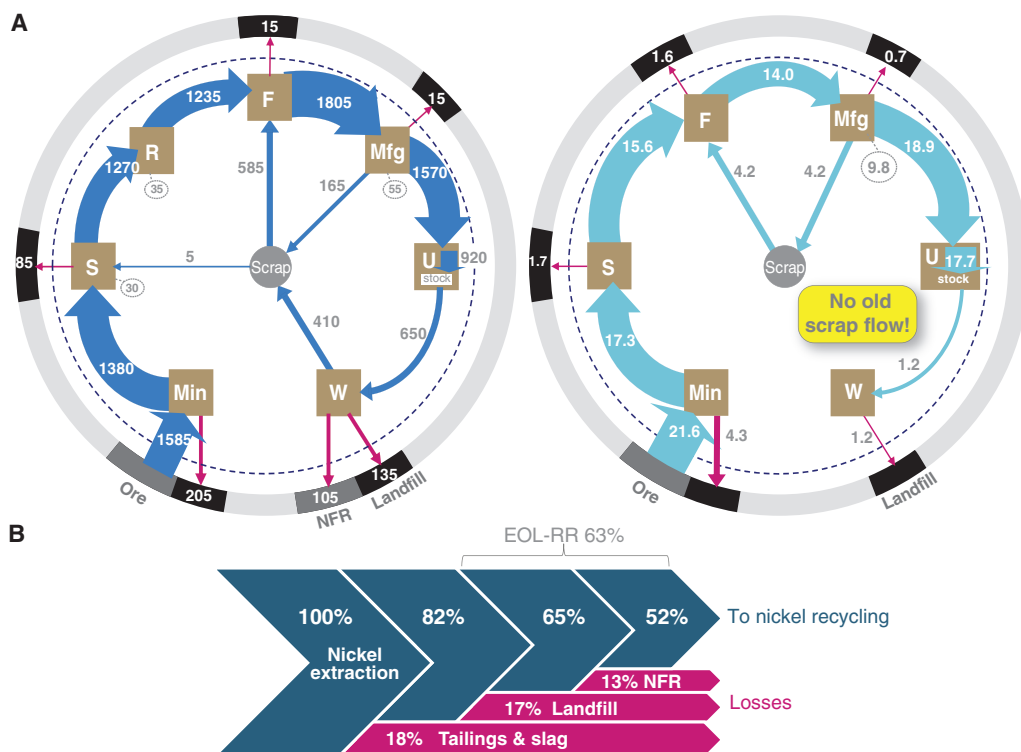


Fig. 2. (A) The global cycles of nickel for the year 2005 [left, adapted from (27)] and neodymium for 2007 [right, adapted from (16)]. The numbers indicate flows of metal within the anthroposphere, in Gg (thousands of metric tons). Flows crossing the dotted line transfer metal to the anthropogenic cycle or vice versa. The width of the arrows is an approximate indication of flow magnitude. Min, mining; S, smelting; R, refining; F, fabrication of semi-products (rolls, sheets, etc.); Mfg, manufacturing; W, waste management and recycling. **(B)** Material efficiencies across nickel's life cycle. Of the extracted nickel, 82% enters fabrication, manufacturing, and end use; 65% enters the recycling processes; and 52% is recycled for another use in which nickel's properties are required (functional recycling). Losses across one life cycle amount to 48%. EOL-RR, end-of-life recycling rate; NFR, nonfunctional recycling.

incorporation as a trace constituent into a recycled stream of iron or copper alloys (Fig. 2B). This confirms the results of Markov chain modeling, which shows that a unit of the common metals iron, copper, or nickel is only reused two or three times before being lost (28–30), gainsaying the notion of metals being repeatedly recyclable.

Product Recovery and Recycling Technology

An engineer or scientist instinctively thinks of technology when the topic of recycling is raised, but it turns out that social and cultural aspects are at least as important, perhaps more so (31, 32). Metal price is a key driver directly affecting collection and processing efficiencies (1, 5). Extensive manual disassembly of discarded electronics is typically not economically feasible in industrialized countries but may be advantageous in emerging economies such as India and China (17, 33). Figure 3 shows the main steps involved in recycling, the key perspective being that the overall efficiency is the product of the efficiencies at each stage. As with a chain, the weakest link controls the performance of the system. The figure also shows the associated recovery and recycling efficiencies for nickel and neodymium across all end-of-life products, as well as the specific cases of nickel and rhenium from end-of-life aerospace superalloys. The first stage is collection, which refers to the transfer of an unwanted product from the owner to a suitable recycling facility.

Collection, pre-processing, and end processing. Collection rates vary greatly among different waste streams, depending on price, logistics,

and other factors. Waste of electrical and electronic equipment (WEEE), in contrast, often has relatively low collection rates despite legislative efforts. In the European Union, 25 to 40% of WEEE is collected and treated in the official system (34), the rest being discarded into municipal waste, exported as used products or scrap, or otherwise lost. Current WEEE legislation in the European Union and Japan focuses on mass recovery, which favors steel and base metals used in large quantities, whereas precious and specialty metals, found in small electrical and electronic equipment, are often not recovered (35, 36). Considering this situation, as well as the recent debate on critical metals [e.g., (37)], a revision of these priorities seems likely (34).

After collection, the postconsumer metal enters a series of pre-processing steps, including repeated sorting (e.g., manual, magnetic, optical), dismantling, and physical and chemical separation (38, 39). Issues of scale are important here. Virgin materials processing is generally large in scale, using processes underwritten by historically low energy prices. In contrast, recycling is often local, more labor-intensive, and smaller in scale. In such a situation, the monetary returns are often not sufficient to justify the purchase of modern “sense and sort” technologies, and much otherwise recoverable material is lost.

The example of a nickel- and rhenium-containing aerospace superalloy shows how price, material combinations, size, and shape can drive the efficiency (Fig. 3). One company estimates that collection rates of these superalloys are around

90% because of their high value and the favorable logistics of a relatively small industry (40). Around 80% of the scrap is in solid pieces that can easily undergo grade-specific identification and recycling. The other 20% is in the form of turnings and other small fractions and can be sent to a stainless steel smelter. This translates into an 81% efficiency for nickel, which is required in both the superalloy and stainless steel, but only a 68% efficiency for rhenium (Fig. 3). Similarly, neodymium may be collected at a rate of 30% from electronics or magnets, but with no element-specific recycling technology existing at present, its overall recycling efficiency is near zero and it will either be discarded or become a trace element in recycled metal.

After pre-processing, the material will be sent to a smelter or other thermochemical facility where processing has been optimized (end-processing). In most cases, these are primary smelters, although some facilities—including electric arc furnaces in steel production as well as smelters processing electronic wastes for the recovery of precious metals, copper, and some specialty metals—specialize in processing secondary metals. As Fig. 1 shows, some metals have fairly high overall recycling rates, generally because they are used in large, easy-to-identify applications such as steel beams or lead batteries, but half or more of the metals face the larger challenge of the recycling sequence and its typical efficiencies.

Recycling technology. Collection efficiencies are related to social and governmental factors, but separation and sorting efficiencies relate to

recycling technology. It is unfortunate from a materials perspective that, for reasons of scale and economics, often only the more basic technologies (shredding, crushing, magnetic sorting) are routinely applied, whereas more advanced technologies (such as laser, near-infrared, or x-ray sorting) are limited to selected recycle streams. Disassembly and liberation of materials is often challenged through product design [e.g., laminated permanent magnets in computers (41)].

Although there are notable examples of innovative recycling technologies, many in demonstration mode, much more attention needs to be paid to modernizing and upgrading existing generic approaches if overall efficiencies are to become higher than they are now (1). Such a modernization could go hand in hand with an international division in labor, as is common practice in manufacturing processes. The best-of-two-worlds approach suggested for electronics recommends taking advantage of the low labor costs in developing countries for manual disassembly, and the high efficiency of specialized smelters, typically located in industrialized countries, for end-processing (42). An encouraging example is Peru, which combines formal and informal collection channels for discarded computers: Single materials such as copper, steel, and aluminum are recycled domestically, while a portion of the complex and valuable printed circuit boards are exported to an advanced smelter in Germany (43). However, some of the boards also go to China for informal recycling, with all the associated potential environmental implications (44).

Improved recovery and recycling performance has occurred here and there in recent years, especially when high-value metals are involved.

The recent spike in rare earth prices has accelerated research into recycling technologies for specialty metals, particularly in Japan after China had briefly cut off its supply of rare earths (45, 46).

State-of-the-art pre-processing facilities are often still optimized for mass recovery, at the expense of recovery of precious and specialty metals. Targeted disassembly prior to shredding could substantially increase the recovery of precious metals from WEEE (47, 48). A Japanese study estimates that additional separation steps in the collection and presorting of small WEEE have the potential to increase gold recovery from the current 26% to some 43%, tantalum up to 48%, and gallium up to 30% (49). And, sometimes, scarce metals can be replaced by more common metals with only modest loss of product performance. Examples are aluminum-doped zinc oxides substituting for indium tin oxides in liquid crystal displays (50) and various compounds replacing rare earths in capacitors (51).

Thermodynamics. Thermodynamics is an ultimate limitation at the final processing stage (38, 39). Few metals are used in pure form; rather, most are components of alloys or other mixtures. When these materials undergo reprocessing, some elements will be reprocessed to their elemental form (e.g., copper), but many will be reprocessed in alloy form [e.g., nickel, tin (52)]. The reason lies in the often similar thermodynamic behavior of alloying elements that make their separation either very energy-intensive or essentially impossible. This is illustrated by the element radar chart in Fig. 4 (53, 54), showing the behavior of impurities during the metallurgical processing of base metals. Elements distributing to the slag and gas phase can sometimes be ex-

tracted in subsequent steps. Elements remaining in the metal phase cannot be separated, with the exception of copper and lead smelting, where consecutive processing steps allow for the removal of the alloying elements (a fact benefiting the recovery of precious metals from electronic waste) (55). The iron metal phase retains both harmful tramp elements (copper, tin) and benign alloying elements (nickel, molybdenum, cobalt, and tungsten). Unless these elements are required in specialty steels, the steel serves as a sink for these valuable and potentially critical elements from which future recovery is basically impossible. The removal of impurities is a much bigger challenge for aluminum (5, 53, 56) and magnesium (54) than for other base metals. Manganese, for example, used in the 3000 series of aluminum alloys, is retained in the metal phase during remelting, producing a melt that would be unsuitable for reuse in any other Al-based system. Unless the 3000 series alloys were separated prior to remelting, the resulting metal would be unsuitable for 95% of all aluminum applications (53). Similarly, lead remaining in copper's metal phase reduces copper's conductivity, making it unsuitable for use in electrical applications (55). This unavoidable circumstance needs to be part of every product designer's knowledge so that metal combinations that cannot be successfully recycled will be minimized. It also highlights the importance of efficient separation during pre-processing steps.

Addressing Future Recycling Challenges

It seems mundane at first telling, but the activity with the greatest potential to improve metal recycling is collection. Such an effort is not so important for iron, copper, or lead, which are typically used in forms that make them easy to identify and reprocess, but is absolutely crucial for the vast majority of metals, used in small quantities in highly mixed products. Collecting discards with high efficiency and with proper care (to avoid mixing that would frustrate later processing) is largely an issue of behavioral habits and incentives, as well as initiatives such as required recycling deposits on consumer goods. Collection and reprocessing of many metals is also hampered by the international trade in used products that sends complex products to countries with inadequate recycling facilities (31, 57). The situation clearly calls for international policy initiatives to minimize the seemingly bizarre situation of spending large amounts of technology, time, energy, and money to acquire scarce metals from the mines, and then throwing them away after a single use.

After attending to collection, the next challenge is to involve the designers of future products in choosing material combinations with recycling in mind. Only designers can reverse the current trend of greater material mixing, but current designs are actually less recyclable than was the case a few decades ago (1). Warnings regarding the increasingly dissipative use of metals are not

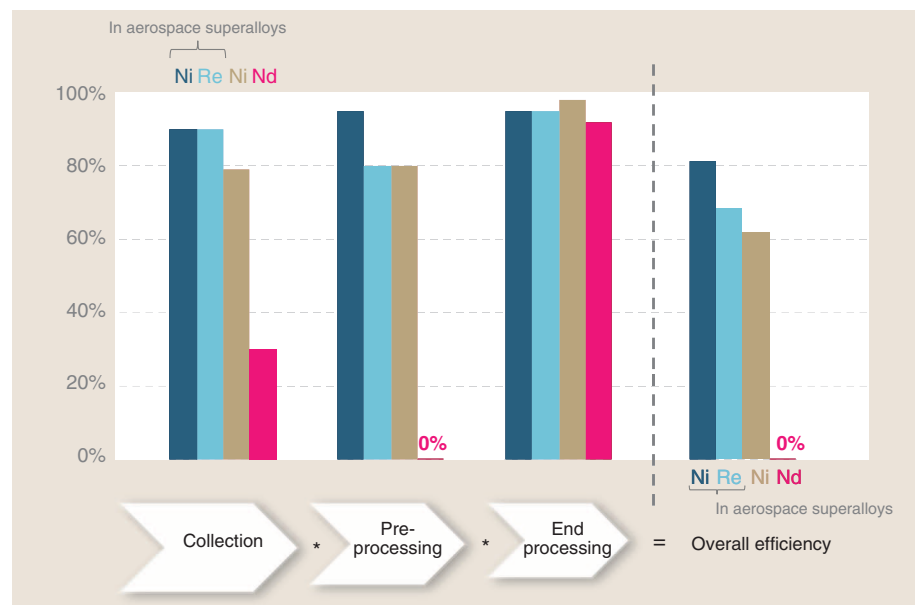


Fig. 3. The sequence of the main steps involved in the recycling of metals. Typical efficiencies are shown for nickel and rhenium in superalloys used in jet engines (40), nickel overall, and neodymium. Collection and end-processing rates for neodymium are estimates shown for illustrative purposes only.

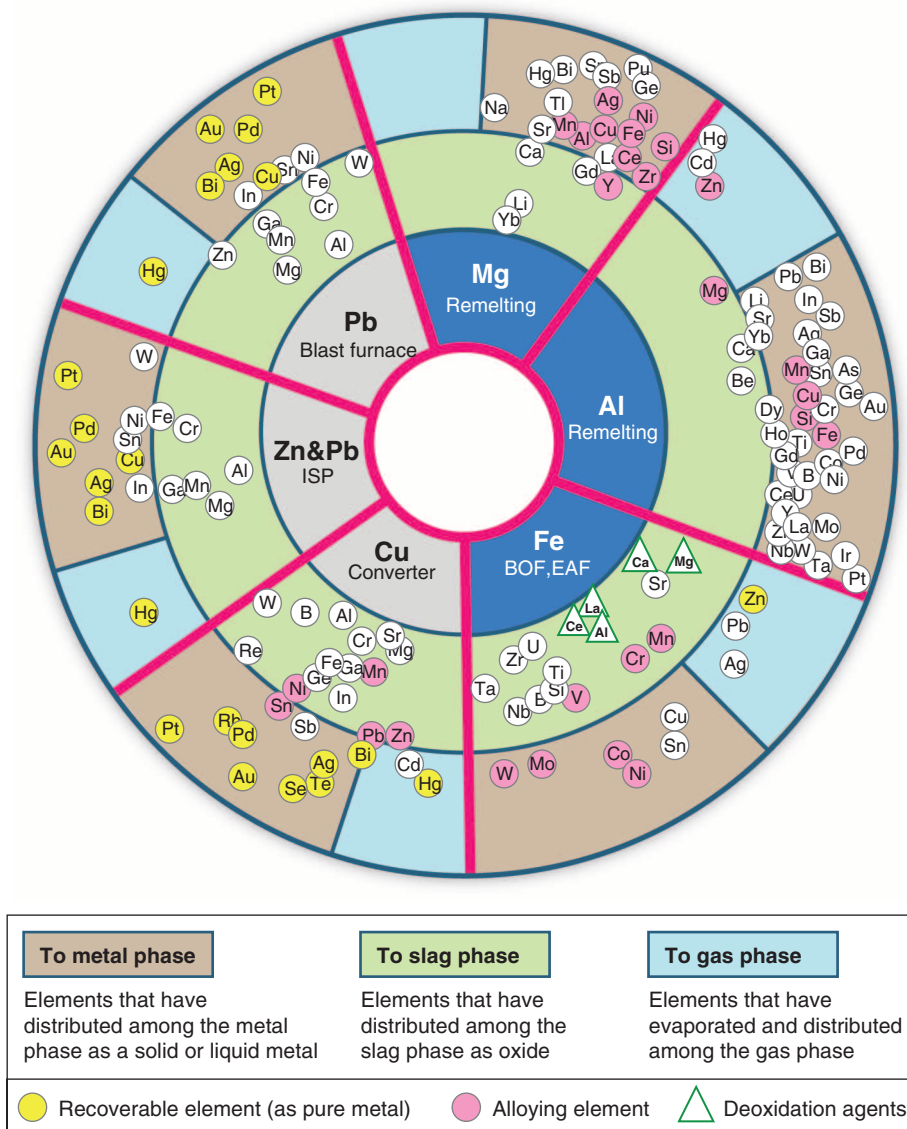


Fig. 4. An element radar chart showing the distribution of alloying elements among metal, slag, and gas phases during thermochemical reprocessing (54). BOF, basic oxygen furnace; EAF, electric arc furnace; ISP, imperial smelting process.

new (3), but applications such as nanomaterials and microelectronics generally introduce major recycling challenges. Ideally, an information feedback loop to materials scientists and designers would emphasize the consequences of complex designs on the recyclability of products (58, 59), leading, for example, to a redesign of alloys to accommodate more scrap (56).

The final frontier is improved recycling technology. It is not much of an exaggeration to say that we manufacture modern products with the best possible technologies we can devise, but generally recycle them with relatively basic approaches. This situation has evolved from a lack of incentives in many directions—little to no support for implementation of new recycling technologies, the unfavorable image of the scrap yard, the frequent specification of virgin material by

manufacturers, and sheer lack of knowledge as to the elemental composition of modern products. It is true that recycling is often limited by unfavorable economics, but it is equally true that those economics reflect a lack of attention to design for recycling and a reluctance to invest in the improved separation and sorting equipment that has emerged within the past decade. It is time that corporations, universities, and governments work together to transform the state of today's metal recycling by demonstrating the need for continuing research on improved technologies, the potential benefits of deployment of the improved technologies now available, and the promise suggested by regulatory and financial initiatives that speak to these challenges.

From the standpoint of the sustainability of metals, the world is at a crossroads. After mil-

lennia of products made almost entirely of a handful of metals, modern technology is today using almost every possible metal, but often only once. Few approaches could be more unsustainable. If as a global society we can collect and reuse almost everything, design products with optimized recycling in mind, and use transformative technology to make the whole process exemplary, we will be helping to ensure that the materials scientists of the future have for their use the full palette of the wonders of the periodic table, and thereby provide society with increasingly innovative and remarkable products.

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REVIEW

Valorization of Biomass: Deriving More Value from Waste

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Most of the carbon-based compounds currently manufactured by the chemical industry are derived from petroleum. The rising cost and dwindling supply of oil have been focusing attention on possible routes to making chemicals, fuels, and solvents from biomass instead. In this context, many recent studies have assessed the relative merits of applying different dedicated crops to chemical production. Here, we highlight the opportunities for diverting existing residual biomass—the by-products of present agricultural and food-processing streams—to this end.

Times are rapidly changing. Who could have imagined that in 2012 a commercially viable venture would involve shipping ~200,000 tonnes (t)/year of household waste from Italy to Rotterdam for use as a feedstock for electricity generation in Dutch power plants with overcapacity (1)? Waste is lucrative business or, as they say in northern England: "Where there's muck there's brass." Since the early 1990s, attention has been diverted from waste remediation to waste prevention, with the emphasis on applying the principles of "green chemistry" (prevention is better than cure) (2). Now the focus is moving toward exploiting those wastes that are largely unavoidable.

In its most general sense, the term "waste" covers any organic material apart from the primary material for which the plants were originally grown (e.g., corn stover from maize or lignin from paper pulping). Nearly all wastes currently have some value—for instance, stover for improving the soil in the fields, or lignin as a fuel to power paper mills. Here, we concentrate on ways of getting higher value from the waste,

particularly via conversion to chemicals. However, making a commercial case for such a process must necessarily include the cost of replacing the original function of the waste—for example, powering the mills with hydroelectricity. Indeed, one can quantify the value of different "waste valorization" strategies (Table 1).

Because the sources of waste are so diverse, it is convenient to consider the chemistry in terms of four source-independent categories: polysaccharides, lignin, triglycerides (from fats and oils), and proteins. As explained later, lignin is challenging to break down into chemically useful fragments. By contrast, pretreatment of polysaccharides, triglycerides, and proteins can lead to their constituent building blocks: monosaccharides, fatty acids plus glycerol, and amino acids, respectively. There are several recent specialized reviews on the conversion of biomass to chemicals (3–6). However, exploiting waste in a profitable way is a highly multidisciplinary problem; therefore, we outline here recent developments for a wider audience with the emphasis on optimizing the valorization of the various components of residual biomass.

Waste is perhaps a concept even broader than the definition above, because it applies to any biomass-derived by-product for which supply greatly exceeds demand. For example, glycerol can be a valuable chemical, but it is being gen-

erated in increasing quantities by the biodiesel industry and could become a "waste." By applying even a crude valorization analysis, one finds that conversion of glycerol to the chemical epichlorohydrin is economically attractive compared to the alternatives, because the value of this conversion is 3 times that of conversion to transportation fuel and 10 times that of burning to generate electricity—hence Solvay's recent commissioning of a new 100,000 t/year epichlorohydrin plant based on glycerol in Thailand (7). In the longer term, glycerol could become a platform molecule leading to many different fine chemicals, but the establishment of such platforms will require a much more mature bio-based chemical industry.

Most biomass waste is a complex and variable mixture of molecules, and separation becomes a key issue. An added complication is that some of both the bio-waste and the materials to be separated are solid; therefore, separation frequently involves organic solvents. If bio-based chemical production is to become self-sustaining, those solvents must also be bio-based and cannot, in the long term, be derived from crude oil. In addition, bio-based solvents would be highly useful materials in their own right. If such solvents can also function as fuel additives and platform chemicals, one would have the basis for a genuinely robust technology (8).

Some of the processing of petrochemical hydrocarbon feedstocks involves the introduction of oxygen-containing functional groups by, for

Table 1. Approximate valorization of biomass waste for different uses* (48, 58).

	Value (\$/t biomass)
Average bulk chemical	1000
Transportation fuel	200–400
Cattle feed [†]	70–200
Generating electricity	60–150
	Cost
Landfill	–400

*Taken from (48) apart from data for cattle feed. The values are based on costs in the Netherlands, but the order of the values is likely to be similar across the developed world. [†]Data from (58); this range of values depends on the quality of the feed.

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example, aerobic oxidation. In biomass, oxygen- and nitrogen-containing functional groups are already present in the feedstock. Therefore, direct conversion of biomass to oxygen- and nitrogen-containing platform chemicals should be more attractive than reductive defunctionalization of the biomass followed by conventional refunctionalization of hydrocarbons. Therefore, it is generally preferable to perform redox-neutral conversions of biomass.

The Size of the Opportunity

Waste constitutes an enormous potential resource: hundreds of megatonnes (Mt)/year [i.e., $>10^8$ t] across the world. Therefore, a bio-based economy must be established on a corresponding scale. The starting point has to be large-volume chemicals—e.g., lubricants, surfactants, monomers for plastics and fibers, and industrial solvents—because they have the potential to make a substantial impact. Merely targeting fine chemicals, although economically attractive and important, would have a negligible impact on sustainability of chemical production because the demand for such chemicals is small. By contrast, platform and bulk chemicals could satisfy a sizable proportion of industrial output.

The largest waste source for carbohydrates and lignin is from lignocellulosic biomass residues, which are estimated to exceed 2×10^{11} t/year worldwide (9). These residues can be separated into two categories: (i) residues left in the field directly after harvest of crops and (ii) residues separated from the product as it is processed. Although the field residues cannot really be described as waste—because soil quality and crop yield are decreased by their removal—the process residues are waste products that are normally burned and could be converted to small molecules.

The two highest-volume process residues are rice husk and sugarcane bagasse. For every 4 t of rice harvested, 1 t of husk is produced, amounting to 120 Mt of rice husk per year. Of this, only 20 Mt is currently used, leaving 100 Mt that could be converted into fuels or chemicals (10). Another high-volume process residue is bagasse, with each tonne of sugarcane yielding 135 kg of sugar and 130 kg of bagasse, resulting in 220 Mt of bagasse per year (11, 12). Most sugar mills burn the bagasse to recover energy but, with improvements to energy efficiency, there could be a large excess available for conversion to platform chemicals (13).

Solid municipal waste is another potential waste stream that could be converted to chemicals, as a large proportion of it is made up of paper and organics (24% and 38% in the United States) (14). Globally, enough waste paper is generated annually to produce 65 Mt of cellulosic-based ethanol (15). Unsold or unused food in developed countries constitutes another biomass waste stream, because liability issues prohibit donations. Solid municipal waste also contains other potential sources of chemicals such as polystyrene and other plas-

tics, but these will not be considered here, as they are ultimately derived from crude oil and are therefore not sustainable feedstocks in the long term.

Lignin and Aromatics

Many key commercial chemicals are aromatic compounds, ultimately derived from petrochemical feedstocks. Lignin is the only large-volume renewable feedstock that comprises aromatics. It is an amorphous cross-linked polymer that gives structural integrity to plants, making up 25 to 35% of woody biomass (16), and can be viewed as a disordered polymer of phenylpropanolic units linked by ether and C-C bonds. Thus, depolymerization of lignin is an alluring route to an important functionality class.

Unfortunately, this route is deceptive. Despite extensive research, there are very few reports of efficient ways of recovering such aromatic products. The only notable commercial process has been the production of vanillin from lignosulfonates, a by-product of the sulfite pulping industry. The maximum yield of vanillin from the optimized industrial process is only about 7.5% by mass (17), and the process struggles to compete with the petrochemical routes to vanillin (18). Currently most lignin is used as an energy source in the pulping industry, and there is no obvious route to valorization beyond the energy route, although new catalysts for lignin conversion are being discovered (19, 20) and the situation could change soon. There is clearly an opportunity for new thinking.

Although there is still no convincing route to single aromatic feedstocks from lignin, there have been important developments in the production of aromatics from nonaromatic biomass sources. It is now possible to obtain totally bio-based styrene from butadiene produced from bio-ethanol or bio-butanol (21) or even directly by fermentation (22). Particularly interesting is recent work by the companies Gevo (23), which has been producing para-xylene from bio-isobutanol, itself produced by fermentation of

sugars derived from cellulose, and Virent, which has been using a three-step chemocatalytic process for paraxylene production (24, 25). Paraxylene is an important aromatic feedstock that is currently oxidized to terephthalic acid (TA) via a surprisingly green aerobic oxidation (26). TA is then condensed with ethylene glycol to make polyethylene terephthalate (PET), the polyester used for clothing and beverage bottles on a huge scale, ~ 40 Mt/year.

Now Gevo has teamed up with the Japanese chemical giant Toray to demonstrate the production of totally bio-based PET from bio-based paraxylene and ethylene glycol (27). Most notably, Coca Cola, Pepsico, and Heinz, some of the largest users of PET bottles, have expressed a commitment to switch to bio-based bottles as soon as practicable (28). Such companies have the necessary market share to drive real innovation in bio-based commodities.

Bulk Chemicals from Carbohydrate Residue

The most abundant prospective feedstocks for sustainable liquid production are carbohydrates, which, together with lignocellulosic biomass, make up most biomass residue. Such feedstocks could provide the basis of biorenewable chemical production without the need to allocate land for specific crops dedicated to this aim, although this conflict is small when compared to the food-versus-fuel problem because the scale of production of chemicals is dwarfed by that of fuels (Fig. 1).

In the first stage of any conversion, the cellulose and hemicellulose must be broken down into their constituent hexose and pentose sugars. The chemistry of this conversion involving hydrolysis with mineral acids has been known for over 100 years and was implemented for production of ethanol from wood during World War II, when cost was not the major constraint. In peacetime, the products were too expensive compared with their low-cost petroleum analogs, and some of the by-products were not marketable (29). Interest in conversion of biomass to sugars

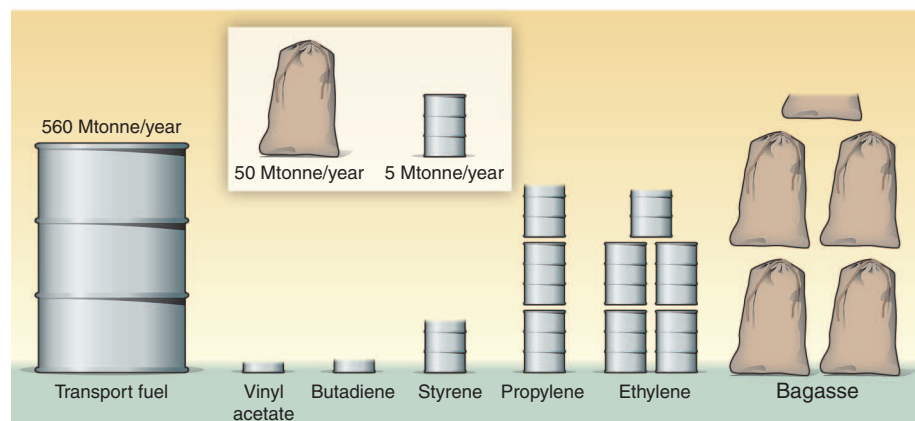


Fig. 1. Schematic diagram to compare the scale of chemical products in the United States and of waste bagasse worldwide, based on 2010 figures (59, 60).

was renewed in the mid-1970s by concerns over security of fuel supplies, and now the conversion is high on the scientific agenda. The key barrier is the efficient recycling of the acid. The current field appears to be led by three companies: BlueFire Renewables, Virdia, and Renmatix. The BlueFire and Virdia processes are similar, the main difference being the recovery step of the mineral acid. Renmatix, by contrast, uses a two-step hydrolysis in high-temperature water for the selective recovery of pentoses from hemicellulose and hexoses from cellulose. All three processes claim to be economically competitive with production of sugars from traditional agricultural sources such as sugar cane. For example, the Virdia process has been quoted (30) as producing sugars at 0.25 \$/kg compared to 0.45 \$/kg for cane sugar.

Licella has developed a catalytic hydrothermal technology to convert corn stover, sugar cane trash, grasses, and farm waste to BioCrude, which can be readily converted to biofuels (31). The combination of catalytic hydrolysis, dehydration, and oxidation results in a very stable crude oil substitute with less than 10 weight % (wt %) oxygen and more than 8 wt % hydrogen. Both ethanol and butanol can be produced by fermentation of the sugars obtained from residual biomass with fermentations similar to those used for first-generation bioethanol today. Such biofuels will be needed where energy needs cannot be met by electrical means, as in the case of aviation fuels (e.g., the 77.2 Mt of jet fuel used in the United States in 2008) or remote sites with low population, and biomass-based liquid fuels could be the only sustainable option. By contrast, the volume of gasoline is so enormous that only a small portion of it could be replaced by biofuels even with a third generation of bioethanol. We believe that current agricultural waste streams could easily be used for smaller-scale applications, without requiring discrete energy crops and simultaneously reducing problems with waste disposal. The utilization of biomass for the production of carbon-based consumer products will become a reality at the latest when all of the economic fossil fuel resources become exhausted, so that most of the research money already spent on biofuels will still benefit later generations. For example, the production of ethylene and propylene—two of the most important seven basic industrial chemicals—from bioethanol would require about 30% of the currently farmed land, which perhaps could be dropped to a sustainable level (less than 2%) with second- and third-generation bioethanol tech-

nologies. The recent rise of shale gas (32, 33) gives us a welcome reprieve to develop these technologies, but this gas is also a finite resource and not a long-term solution to sustainable chemical production.

Beyond application as a fuel, fuel additive, or solvent, ethanol can be dehydrated to produce the versatile commodity compound ethylene (34), which in turn can be oxidized to ethylene oxide and ethylene glycol or polymerized. For example, the Brazilian company Braskem (35) is already producing renewable polyethylene from ethanol on a commercial scale. A key point is that these are major chemicals in the petrochemical economy and, therefore, production from ethanol can easily be incorporated into existing supply chains. Ethanol does, however, have drawbacks

promise as platform organic molecules. Furfural can be the starting material for the synthesis of a series of derivatives including furfuryl alcohol, furoic acid, furan, tetrahydrofuran, 2-methyl-tetrahydrofuran, and related resins.

5-HMF is an even more attractive platform (37) (Fig. 2). It can easily be converted into dimethylfuran, which has applications as both solvent and transportation fuel (38). It can also be converted to furan dicarboxylic acid (39–41), which has the potential to become a major bulk chemical because it can be copolymerized with ethylene glycol to make a renewable polymer with properties similar to those of the PET polyesters used for textiles and packaging. Thus, 5-HMF combines the two key criteria for valorization of biomass: It retains a reasonable proportion of the original chemical complexity, and it can also be converted to high-tonnage chemicals.

5-HMF can also be converted to gamma-valerolactone (GVL) via levulinic acid (Fig. 2). The beauty of this route is that the coproduct of levulinic acid is formic acid, which can act as a source of the hydrogen needed for the subsequent conversion to GVL (42, 43). GVL is a platform molecule in its own right, which can even be converted to adipic acid, the precursor for Nylon, opening up another “bio-route” to a major chemical.

Fermentation of carbohydrates can also lead to lactic acid, used primarily in the production of poly(lactic acid), a biodegradable plastic. The properties of poly(lactic acid) are sufficiently similar to those of petroleum-derived polymers

that it can replace them in a wide range of applications (e.g., packaging, fibers, and foams) (44). The relevance in the context of this review is that this technology has already been commercialized on a scale of $>10^5$ t, thereby demonstrating that such an approach can be both sustainable and profitable (45).

Protein Waste to Platform Chemicals

Substantial amounts of protein-containing waste are generated in the production of foods and beverages. Examples include vinasse (from sugar beet or cane), distiller's grains with solubles (from wheat or maize), press cakes (from oil seeds like palm and rapeseed), fish silage, protein from coffee and tea production, and agricultural residues from various crops. For example, poultry slaughterhouses produce large quantities of feathers with a crude protein content of more than

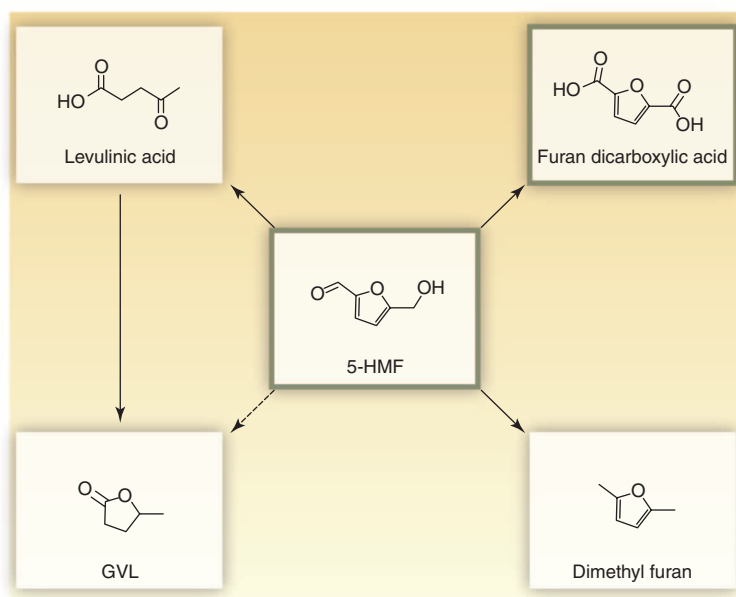


Fig. 2. 5-HMF as a platform chemical.

as a transportation fuel; it is hygroscopic and expensive to purify after fermentation, because fractional distillation is limited by the low-boiling azeotropic mixture incorporating 5% water. These problems are focusing interest on butanol as an alternative fuel. The ready availability of butanol would have important implications for chemical production because it would make the conversion of butanol to butenes and other chemicals commercially attractive.

From a chemical perspective, a downside to fermentation is that the alcohols have lost much of the chemical complexity of the original sugars, which then has to be rebuilt to make the target chemicals. By contrast, more of the complexity can be retained after acid-catalyzed dehydration of pentose and hexose sugars, which yields furfural and 5-hydroxymethyl-furfural (5-HMF) (36), respectively (19). Both compounds show

75% w/w (46), 65% of which consists of nonessential amino acids. Similarly, the production of shrimp meat generates large amounts of protein waste together with the carbohydrate chitin (47). Much of this protein is currently processed as animal feed but, applying Table 1, it would have more value as a feedstock for commodity organic compound production. This is an area that has been discussed rather less than carbohydrates and lignin. In the ideal scenario, the essential amino acids contained in this protein waste could be used as animal feed and the non-essential amino acids, with no real value for food or feed, as chemical feedstocks. This would again circumvent the fuel-versus-food issue.

Another, potentially enormous source of protein waste that could be exploited in the future will be the by-products from the production of biofuels. It is generally agreed that, in the long term, this will largely involve second-generation biofuels from lignocellulosic biomass and nonedible triglycerides. It has been estimated (48) that if 10% of transportation fuels, currently derived from fossil feedstocks, were substituted by biomass-derived fuels, this could generate 100 Mt/year of protein worldwide. This amount is of the same order of magnitude as the protein requirement of the world population. In an integrated biorefinery, these proteins could form a source of bulk chemicals via a three-stage process: (i) isolation and hydrolysis of the proteins to mixtures of amino acids, (ii) separation of the individual amino acids, and (iii) conversion of the amino acids into bulk organic compounds, which will generally comprise large-volume industrial monomers.

A promising technology for the isolation of proteins from biomass residues involves so-called ammonia fiber expansion (AFEX) pretreatment whereby cellulose is separated from proteins that are solubilized. The protein fraction can then be hydrolyzed. Traditionally, this involved prolonged treatment with concentrated mineral acids at elevated temperatures, leading eventually to the formation of copious amounts of inorganic salts as waste. A milder and more environmentally attractive alternative involves the use of proteases, such as the alkaline protease alcalase, which is widely used in laundry detergents. It has been used, for example, in the hydrolysis of proteins in poultry (46) and shrimp waste (47). A possible drawback of this method is the relatively high cost of the enzymes. Immobilization of the protease could provide great-

er economic viability by enabling recovery and reuse of the enzyme. Furthermore, immobilization would suppress degradation of the protease by autolysis (49).

In the second stage, individual amino acids must be isolated from the protein hydrolysate. Standard methods generally involve fractionation on the basis of physicochemical characteristics such as molecular size, charge, and hydrophobicity. Here, there is a clear need for innovation. Certain amino acids could perhaps be selectively converted—by enzyme-catalyzed decarboxylation, for example—to products that can be more easily separated. In one approach, protamylase, an amino acid-rich waste from potato starch processing, was used as a feedstock for the pilot-scale coproduction of ethanol and cyanophycin (CGP: a nitrogen storage polymer

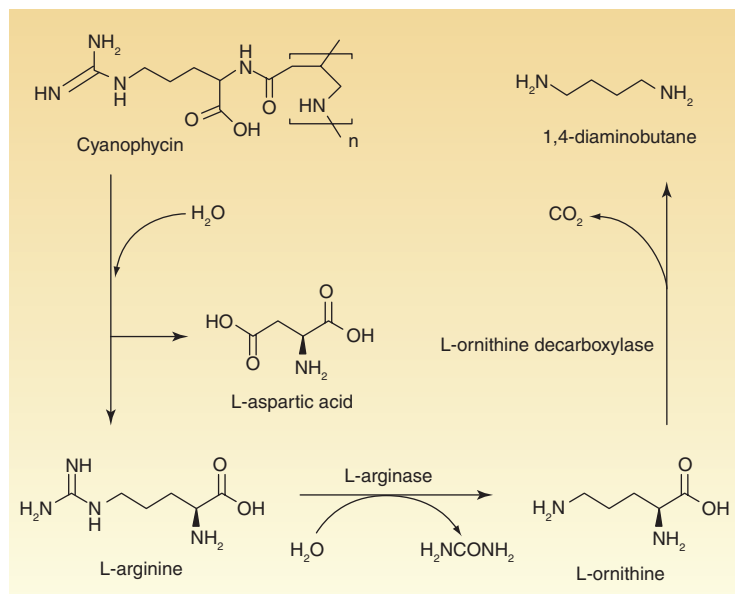


Fig. 3. 1,4-Diaminobutane from cyanophycin.

In the third stage, individual amino acids can be converted into commodity chemicals. To be competitive with petrochemical-based routes, these conversions should preferably involve as few functional group changes as possible and be close to redox neutral. Obvious candidates would be, for example, a variety of nitrogen-containing compounds that retain the amino group of the amino acid (51). The nonessential amino acid, L-arginine, produced by hydrolysis of cyanophycin can be converted in two enzymatic steps—hydrolysis and decarboxylation—to 1,4-diaminobutane (Fig. 3) (52), the starting material for Nylon-4,6.

There is a great need here for out-of-the-box thinking. Chemists are accustomed to thinking of proteinogenic amino acids

as largely chiral molecules that are synthesized from simple starting materials. Now they have to start thinking about how to convert these same amino acids back to relatively simple commodity chemicals in an economically viable manner. An illustrative example is provided by L-phenylalanine, which can be made by enzymatic addition of ammonia to cinnamic acid. However, the fermentative production of L-phenylalanine from biomass has become so efficient that the most economical way to produce cinnamic acid is probably from L-phenylalanine by the reverse reaction. One can also imagine that, at a certain feedstock price and with efficient technology, integration of the deamination with subsequent decarboxylation could afford a bio-based process for styrene (Fig. 4).

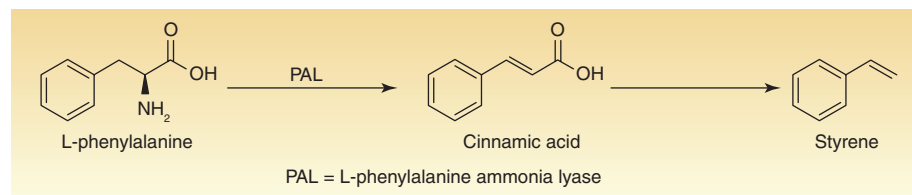


Fig. 4. L-Phenylalanine to cinnamic acid and styrene.

produced *in vivo* by cyanobacteria) by yeast fermentation (Fig. 3) (50). CGP consists of a poly(L-aspartic acid) backbone with equimolar amounts of L-arginine side chains and, because it is insoluble under physiological conditions, it can be easily isolated from the fermentation broth.

Glutamic acid, the most abundant, non-essential amino acid derived from the hydrolysis of many plant and animal proteins, can be converted to a variety of commodity chemicals via initial decarboxylation catalyzed by glutamic acid α -decarboxylase (53). However, not all the routes are sustainable. A life-cycle assessment study (54)

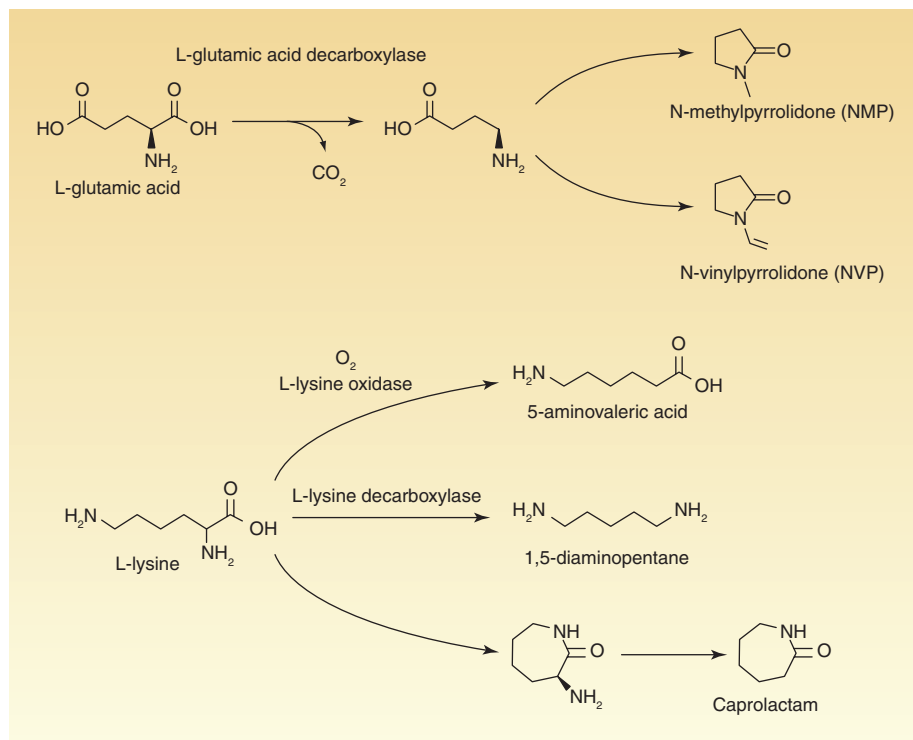


Fig. 5. Possible bio-based commodity chemicals from glutamic acid and lysine.

compared four bio-based commodity chemicals derived from glutamic acid with their petrochemical equivalents (Fig. 5); *N*-methyl- and *N*-vinyl-pyrrolidone had less environmental impact, whereas acrylonitrile and succinonitrile (not illustrated) had more impact than the petrochemical route. The unfavorable enviro-economics of the latter two bio-based products were directly related to the inferior chemistry involved, inter alia, oxidation with hypochlorite.

L-Lysine is another interesting platform chemical that can be converted to a number of industrial monomers, such as 1,5-diaminopentane (55), caprolactam (56), and 5-amino valeric acid (57) (Fig. 5). Although it is an essential amino acid, in the future there may be sufficient overproduction from waste protein to make it an interesting feedstock for organic compound manufacture.

Outlook

These examples are just a few of the possibilities for valorization of the huge amounts of animal and plant residue that we produce each year. The pace of research in this area is accelerating, and chemical manufacturers are showing increased interest in renewable feedstocks. Here, we have focused on agricultural and food waste but, with rapid urbanization across the world, municipal waste is likely to become an increasingly important source of waste biomass. This is particularly likely in economically developing countries where the demand for chemical products is growing and the waste has a higher organic content than in more developed economies.

We have not considered the question of water availability, which may be a limiting factor in the processing of biomass. Fortunately, most of the world's population lives near the coast, and promising results are beginning to emerge in the use of seawater and brine for the processing of biomass. This may indeed be the way forward. However, genuine progress will require a close partnership between chemists and engineers so that new ideas in chemistry can be quickly transformed to meet the needs of our planet's expanding population.

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PERSPECTIVE

Recycling of the #5 Polymer

Marino Xanthos

Polypropylene (PP) is a widely used plastic with consumer applications ranging from food packaging to automotive parts, including car battery casings. To differentiate it from other recyclable plastics, it is designated as #5. Here, the factors contributing to PP recycling rates are briefly reviewed. Considerations include collection and separation efficiency, processing chemistry, and market dynamics for the products derived from recyclates.

In 1970, recycling of plastics from the municipal solid waste (MSW) stream was virtually nonexistent. By 1994, the recycling rate of different types of plastics found in the United States MSW (containers and packaging, durable goods, and nondurable goods) increased to 4.7% (1), and it reached 8% in 2010 (2). Similar growth can also be found in the plastics recycling rates of many Western European countries—and also in Japan since the introduction of government legislation in 1995. The low levels of what is known as “mechanical” recycling (the term excludes incineration with or without energy recovery or conversion into chemical feedstock) reflect the complexity of discarded and used materials and the corresponding issues with their effective separation and cleaning. However, the recycling rate for some individual plastics is much higher. Sorting of commodity thermoplastics used in packaging (e.g., containers and films) is facilitated by using a number identification code from 1 to 7. This is a voluntary system introduced by the Society of Plastics Industry in 1988 with the goal of helping consumers to determine whether certain types of plastics are collected for recycling in their area (2). The system is also in use in countries other than the United States (e.g., Canada and Switzerland). In North America, successful recycling operations mostly deal with #1 and #2 packaging plastics [polyethylene terephthalate (PET) and high-density polyethylene (HDPE), respectively], for which large-scale collection infrastructures exist and commercial applications for the recyclates have existed for some time. For example, in 2010, 28% of HDPE bottles and 29% of PET bottles and jars were recycled (2).

Polypropylene Recycling Overview

Polypropylene (PP), designated as #5, is among the commodity thermoplastics found in the MSW. Its recycling is technically possible and has been practiced for certain single-industrial products and industrial scrap for more than three

decades. Recycling of PP bottles, although increased in 2010 to 35.4 million pounds, is still lower than either PET or HDPE (3). In addition to the need for adequate collection means, the challenge for postconsumer packaging is in separating it from other plastics—including its own many variations—once it arrives at the waste station and beyond. For PP, recyclability is also related to a certain extent to its structural instability during melt reprocessing. PP may be found in other mixed waste streams, such as wire and cable coverings, discarded electronics, automotive shredder residue, and carpets (4). Here, I refer to recycling of PP items in general and not only to the ones that may appear in the MSW.

Polypropylene has several attributes (low density, thermal and chemical stability, stiffness, and low cost) that make it quite appealing for use in food packaging, including microwave-safe containers. These same properties have made PP an excellent choice in a variety of automotive and other industrial contexts. Specifically, it is used in the manufacture of carpets, ropes, automotive parts, car batteries, containers, crates, piping, furniture, consumer electronics, bottle tops, living hinges, laboratory equipment, storage boxes, buckets, medicine packaging, and even banknotes (Fig. 1). Recycled PP products may be the same as the original (e.g., auto parts, pallets, and storage bins) or repurposed to applications with less stringent performance requirements (e.g., bike racks, brooms, ice scrapers, buckets, and consumer items) (5). For example, PP banknotes, when withdrawn from circulation, are granulated and then recycled in household and industrial products such as wheelbarrows, compost bins, and plumbing fittings (6). So far, recycling of food containers to food-contact-grade packaging has not been possible given the stringent quality checks that recycled plastics need to meet under government standards; substantial efforts, however, are still in progress in the United Kingdom (7).

Recycling of industrial scrap from extrusion or injection molding has been a common practice over the years. Recycled PP products may include high-value or lower-value items, as discussed earlier. In general, demand and markets for post-

consumer recyclates appear to be steadily growing. Driving forces include legislated minimum recycled-content mandates, procurement policies, expanded waste collection networks, and improvements in recycling technology.

Restabilization of PP

In general, recycled plastics are less costly than the original materials, unless separation and cleaning (beneficiation) costs were excessive; many recycled plastic items are considered to be useful only for low-value applications. It is common for quality and appearance to degrade with each reprocessing cycle. This is particularly true for polypropylene items, which, due to the presence of tertiary carbon atoms in the polymer backbone, are susceptible to pronounced thermo-oxidative degradation during melt processing and/or during use, as well as photo-oxidative degradation during use.

Plastics usually contain stabilizers that protect the polymer from such degradation. These stabilizers are usually consumed during melt processing and/or leach out during exposure of plastic parts and need to be replaced (through a process termed restabilization) to enhance the processing stability and increase the service life of the recyclate. The molecular weight and/or molecular-weight distribution of the initial polymer can change as a result of chain scission (typical for polypropylene) or cross-linking reactions (typical for polyethylene). Both these types of reactions may lead to irreversible changes in mechanical and rheological properties (8).

In the case of virgin PP, melt processing is only possible in the presence of combinations of a processing stabilizer or a blend of processing stabilizers with different, synergistic modes of action. Typical compounds for this purpose include a combination of sterically hindered phenols with phosphite or phosphonite substituents and an acid acceptor. Restabilization can substantially minimize further melt viscosity reduction that would correspond to an increase of the melt flow rate (MFR) as a result of molecular-weight reduction. Types of additives and their associated concentrations for restabilization can be screened by performing multiple extrusions, which would simulate repeated processing, or long-term exposure of the recyclate to elevated temperatures. Depending on the extent of the polymer degradation during use, different types and levels of restabilization will be required that may increase the overall materials cost of the recyclate; it has been shown, however, that restabilization is the key for successfully recycling PP in a variety of items such as crates and films (8).

Packaging

A few prominent food and beverage companies in the United States are moving on their own to recapture their packaging after its use by their

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PERSPECTIVE

The Challenges of Reusing Mining and Mineral-Processing Wastes

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Mining and mineral-processing wastes are one of the world's largest chronic waste concerns. Their reuse should be included in future sustainable development plans, but the potential impacts on a number of environmental processes are highly variable and must be thoroughly assessed. The chemical composition and geotechnical properties of the source rock determine which uses are most appropriate and whether reuse is economically feasible. If properly evaluated, mining waste can be reused to reextract minerals, provide additional fuel for power plants, supply construction materials, and repair surface and subsurface land structures altered by mining activities themselves.

Mining and mineral-processing wastes—the solid and liquid materials generated after mining and ore processing at or near mine sites (*1*)—have no current economic use. A number of environmental problems are associated with the disposal of this waste, including contamination of streams and lakes (*2*) and pronounced landscape transformation (e.g., stockpiled waste rock and tailings, subsidence basins, open pits, and removal of overburden rock and topsoil) (Fig. 1). Despite several efforts to reduce the amount of waste produced, solid mineral wastes remain one of the world's largest waste streams

(*3*). For example, North America produces more than 10 times as much solid mine waste as municipal solid waste per capita (*4*). Because mineral production continues to be necessary for economic development, the recycling and reuse of mining and mineral-processing wastes are important management strategies now and in the future (*5*).

The origin of mining and mineral-processing wastes is closely related to the formation of the target resource or minerals. For example, many coal deposits exist in subsided regions resulting from mountain formation; hence, the overlays of coal resources are generally not very thick and consist of relatively inactive sedimentary rocks. In 2010, worldwide total coal production was about 7273.3 million tonnes (Mt), with an estimated waste of about 1454.7 Mt due to coal production (*6*). Of this waste, up to 100% (total waste with no production of prospective minerals) may be due to the mining or extraction method. Wastes produced during coal preparation (removal of undesired materials from coal through coal washing, crushing, screening, and dewatering) may reach 10 to 30% of raw coal; most of these wastes are in slurry form as a result of the washing process. The final form of waste can be detrimental

to the feasibility of reuse and recycling because it dictates the cost of further processing.

Mining and mineral-processing wastes consist of rocks, soils, oil sands, and loose sediments. The mineralogical and chemical characterizations of mining wastes are useful in forecasting geotechnical properties (particle size and structure, plasticity, bulk density, dry density, shear strength) of the waste and the leachability of potentially harmful compounds. The mineralogical composition of the processing wastes can be heterogeneous because of the deposition of wastes from the processing of different mine sources, yielding a range of physical and chemical properties. For example, the mineral composition of tailings from metal and nonmetal mines in China is divided into eight broad types (*7*).

The most important mineralogical considerations are those that influence mineral recovery, decontamination, acid rock drainage, and processes that affect sediment strength and cohesion. The concentrations of toxic elements and metalloids such as Cd, As, Hg, Cr, and Pb are highly variable, but if present in sufficient quantities, they may inhibit plant growth or degrade water quality (*8, 9*). Methods such as mechanical separation, chemical carbonation, and hydrothermal mineralization (*10*) can remove some of these toxic elements, but may also in some cases mobilize metals in groundwater and surface waters through oxidation.

The reuse of mining and mineral-processing wastes may minimize the environmental impacts related to disposal; however, some reuse and recycling measures may actually cause new and serious environmental problems. The overall environmental costs can be determined by various approaches such as ecological risk assessment, life cycle assessment, sustainability operations assessment, and ecological footprint estimates (*3, 11, 12*). Economic cost-benefit analysis, however, is the ultimate driver in terms of the feasibility of a specific reuse technology. If the costs of final target material extraction or mine waste reuse method are economically prohibitive, then even the most ecofriendly process methods will be difficult to implement without regulation or government subsidies.

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One approach to minimize cost is to improve waste processing efficiency, which depends on the optimization of the resource allocation to minimize environmental effects while maximizing the quantity of wastes processed and the associated benefits. Avoiding waste in the first place is the most favored means of increasing waste processing efficiency because it has the least environmental impact and possibly involves the least energy spent on waste disposal; however, it is also the most difficult to accomplish. The use of solid mining waste as backfill and stabilization material in underground coal mining is potentially a good way to increase efficiency, but the trade-off is not straightforward because of the energy costs related to additional tunnel operations to move the material, as well as the need to create open space for temporary waste storage and management (13).

Residual mining wastes after reuse or resource recovery are typically discarded at specific sites such as tailing ponds. If wastes are not disposed of properly, wastewaters, especially from hydrocarbon wastes, can enter streams and potable supply wells. The primary goal for disposal of mining and mineral-processing wastes should be to ensure that the waste remains physically, geographically, chemically, and radiologically stable and inert, and if this is not possible, the wastes must be isolated and prevented from interacting with the ecosystem (14). Reuse of discarded mine waste, referred to as tailing recovery, helps reduce exposure of waste to the environment and in some cases can maximize target mineral efficiency (15). For example, waste rock or coal slime generated after washing processes may contain carbon with calorific values of 3350 to 6280 kJ/kg, which can be remixed with coal for additional power generation (16). As above, the reuse of mine tailings or coal slimes also may have potential negative environmental impacts, such as increased emissions of nitrogen dioxide and sulfur dioxide.

Considering the factors that dictate when and where mining waste reuse makes sense environmentally, economically, or both, there are several examples of successful reuse projects. Waste rocks and iron/steel slags have been used as alternative aggregates for the construction of roads and railroad banks (17), river embankments, dikes, and dams (18). As a result of regulatory policies, mine waste reuse for roadways and parking areas is an accepted

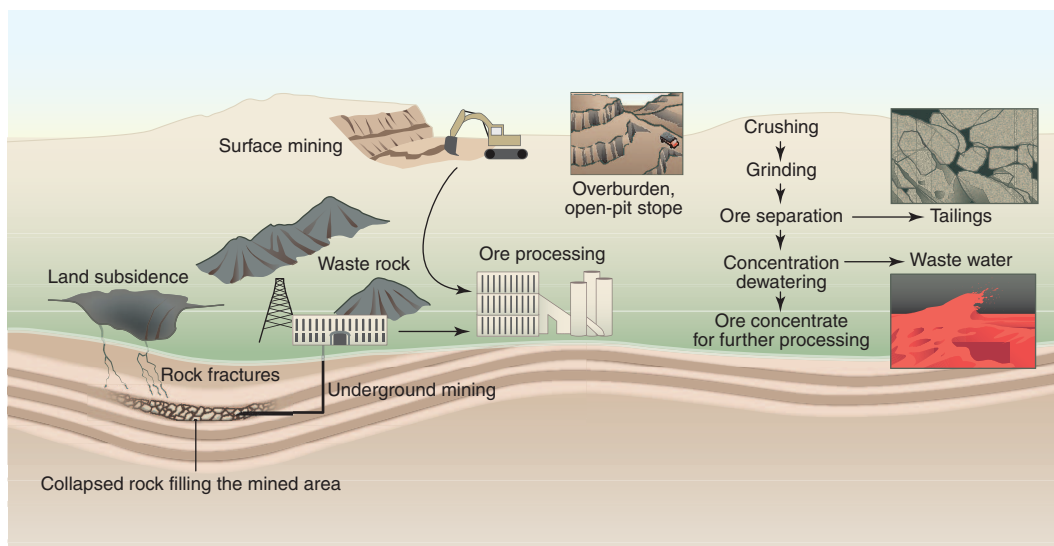


Fig. 1. Waste generated and environmental effects during different mining stages.

practice in China but not in the United States. Waste rock and tailings have also been used as auxiliary source materials for producing building materials such as cement, hollow bricks, concrete, and glass (17, 19–21). Ground subsidence basins induced by mining have also been filled with waste rock and covered with topsoil. The repaired land can then be reclaimed as farmland, grassland, or construction land (11). The waste rock or tailings can also be crushed and mixed with fly ash and cement as backfill in mined cavities, which has the potential to reduce surface subsidence and is a promising method for large amounts of waste reuse (22).

It is difficult to assign a universal method to reuse all kinds of mining and mineral-processing wastes. Each kind of waste has its own appropriate ways for reuse, which even can vary according to local environmental conditions (e.g., proximity to drinking water, depth of mining activity). In any situation where mining and mineral-processing wastes are reintroduced back to the subsurface, efforts must be made to ensure that no pollutants transfer from mining wastes to food or water supplies. Appropriate environmental monitoring and assessment studies should always be included in the reuse design.

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Evolution of Ocean Temperature and Ice Volume Through the Mid-Pleistocene Climate Transition

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Earth's climate underwent a fundamental change between 1250 and 700 thousand years ago, the mid-Pleistocene transition (MPT), when the dominant periodicity of climate cycles changed from 41 thousand to 100 thousand years in the absence of substantial change in orbital forcing. Over this time, an increase occurred in the amplitude of change of deep-ocean foraminiferal oxygen isotopic ratios, traditionally interpreted as defining the main rhythm of ice ages although containing large effects of changes in deep-ocean temperature. We have separated the effects of decreasing temperature and increasing global ice volume on oxygen isotope ratios. Our results suggest that the MPT was initiated by an abrupt increase in Antarctic ice volume 900 thousand years ago. We see no evidence of a pattern of gradual cooling, but near-freezing temperatures occur at every glacial maximum.

The deep-ocean sediment oxygen isotopic composition record ($\delta^{18}\text{O}_\text{C}$), commonly considered to be a record of global ice volume and thus a chronicle of ice ages, also is “heavily contaminated” (1, 2) by the effect of deep-water temperature variability. The contributions of ice volume and temperature to $\delta^{18}\text{O}_\text{C}$ have been debated for more than 40 years [e.g., (1–5)], and definition of the trajectories that each followed during the mid-Pleistocene transition (MPT) has been elusive. Here, we present a high-resolution record of deep-water temperature, which we constructed based on foraminiferal Mg/Ca paleothermometry (see supplementary text and fig. S1). Only one previous deep-sea temperature record extending over the MPT and obtained independently of oxygen isotopes exists, and this is at orbital-scale resolution from the North Atlantic using Mg/Ca ratios from benthic foraminifera (6). However, it has been recognized recently that Mg/Ca ratios of epifaunal benthic foraminifera are affected by carbonate saturation effects (7, 8), requiring a “regional calibration,” and, consequently, the reliability of this approach has been both challenged (9) and defended (10). The increasing interest in applying inverse models to deconvolve temperature and ice volume in climate records [e.g., (11–14)] adds to the interest in separating these signals from the oceanic sediment record using geochemical methods.

Previously, we carried out a calibration study using the shallow-infaunal benthic foraminifera *Uvigerina* spp., showing that it is little affected by bottom-water carbonate saturation, and generated

a low-resolution benthic temperature record for the past 440 thousand years (ky) (15). We now have generated a 1.5-million-year record of deep-sea temperature and sea level with a resolution (≤ 1000 ky) sufficient to define their behavior over the period of the MPT, including a comparison with the climate of the last 800 ky derived from ice-core records (16) and information on changes in ocean carbon chemistry.

The site studied—Ocean Drilling Program (ODP) leg 181, site 1123—is located on the Chatham Rise, east of New Zealand at $41^\circ 47.15'\text{S}$, $171^\circ 29.94'\text{W}$, at a depth of 3290 m (fig. S2). It lies under the Deep Western Boundary Current (DWBC) that feeds the Pacific and comprises Lower Circumpolar Deep Water (CDW), which forms from dense waters sinking around Antarctica, particularly cold waters from the Weddell and Ross seas and Adelie Coast. The modern hydrography of the site shows a slight contribution from North Atlantic Deep Water (NADW) expressed by a small salinity anomaly marker (17). More than half of the flux of cold bottom water entering the major basins of the world ocean does so through the Southwest Pacific Ocean, as the Pacific DWBC (17, 18). CDW is drawn to the surface by upwelling with descending return flows south of the upwelling as Antarctic Bottom Water (AABW) and north of it as Antarctic Intermediate Water. The connection of this site to the regions where deep Antarctic water masses form enables comparison with Southern Ocean and Antarctic atmospheric records and is important for determining the role of the deep ocean in climate evolution.

Benthic $\delta^{18}\text{O}_\text{C}$, deep-sea temperature, and Antarctic temperature. We determined $\delta^{18}\text{O}_\text{C}$ and Mg/Ca on 1650 and 1485 samples of *Uvigerina* spp. (supplementary text, table S1, and Fig. 1). Data are also archived at doi:10.1594/PANGAEA.786205. The $\delta^{18}\text{O}$ data were correlated to an orbitally tuned benthic $\delta^{18}\text{O}$ stack, LR04 (19), to provide an initial time scale. The $\delta^{18}\text{O}$ record of benthic foraminiferal calcite extends from marine isotope stage (MIS) 50, 1550 thousand years ago (ka), to the Holocene (Fig. 1A). It shows features in common with the LR04 stack with stable interglacial values (except for the anomalous interglacials MIS 5, 9, and 11) and lower glacial values before MIS 22.

There are, however, differences between our $\delta^{18}\text{O}$ record and the LR04 stack, in particular over the period thought to encompass the MPT. Benthic $\delta^{18}\text{O}_\text{C}$ shows an abrupt increase from about 950 ka to 870 ka, whereas LR04 shows a more gradual increase to values 0.5 per mil (‰) lighter than for ODP 1123. This is not an artifact of benthic species analyzed, because we have verified this observation by measuring $\delta^{18}\text{O}$ also on the epibenthic species *Cibicides wuellerstorfi* (fig. S3A).

What is the importance of this difference? LR04 is thought to represent a global average from which regional variability has been removed. Nevertheless, LR04 is not an area-weighted stack and is biased to the Atlantic Ocean and eastern equatorial Pacific. Only one site samples the major flow to over half the volume of the world ocean and that is ODP 1123. The LR04 stack is widely used, but to discuss the implications for changing global ice volume we need to delve further. Because of the possible bias in the stack, it makes more sense to compare our site 1123 record with a few other high-quality records from the world oceans. The more abrupt change in $\delta^{18}\text{O}_\text{C}$ is seen in some records but not others (fig. S3, B to D). Some records show a stepped increase in $\delta^{18}\text{O}_\text{C}$ over the period 1000 to 600 ka. If interpreted as ice volume, $\delta^{18}\text{O}_\text{C}$ shows that the first large ice sheet was at MIS 16 in the Atlantic but at MIS 22 in the Pacific, as indicated by ODP 1123. It is likely that the difference of individual sites from each other and from LR04 reflects changes in hydrography, smoothed in the stack.

The benthic $\delta^{18}\text{O}_\text{C}$ record has a temperature component and a seawater $\delta^{18}\text{O}$ component, and the latter may reflect a combination of a global ice volume signal and a local hydrographic signal relating, for example, to changes in proportions of colder low $\delta^{18}\text{O}$ AABW versus warmer high $\delta^{18}\text{O}$ NADW. If our signal may reflect “local” hydrographic changes, those changes are related to Antarctica because deep-water masses at site 1123 originate from waters sinking around Antarctica. Detailed records in addition to those in Fig. 1 at key hydrographic locations would be required to fully assess variability in the global seawater $\delta^{18}\text{O}$ signal. Our assessment is that the hydrographic component at the site of 1123 is small: (i) benthic isotopic depth profiles at the site of ODP 123 are related to the structure of water masses at present and inferred for the past, with no apparent changes in the depths of water-mass boundaries between glacial and interglacial states (17); (ii) estimates of oxygen isotopic composition of seawater ($\delta^{18}\text{O}_\text{w}$) from corals and

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pore water modeling discussed below show global values. Ideally, what is needed is a global array of seawater $\delta^{18}\text{O}$ records that could be stacked to produce a mean ocean $\delta^{18}\text{O}$ signal.

There is also considerable millennial and sub-millennial scale variability in the records, more in Mg/Ca than $\delta^{18}\text{O}_{\text{C}}$ (supplementary materials and Fig. 1, A and B). To aid in comparison, oxygen isotope and Mg/Ca data have been smoothed in the time domain using a Gaussian filter having a total width of 5 ky. The range in Mg/Ca of *Uvigerina* spp. is narrow (~0.8 to 1.3 mmol/mol) but shows clearly a different overall pattern than $\delta^{18}\text{O}$ (Fig. 1B).

Mg/Ca was converted to temperature using the sensitivity previously determined (15) of 0.1 mmol/mol per $^{\circ}\text{C}$. This is based on 56 core top samples that show a statistically significant correlation between Mg/Ca and bottom-water temperature (fig. S4). Results show a temperature range from about 3°C in the warmest interglacial to a reasonably constant value of about -1.5 to -2°C for the glacials (Fig. 1B), which we discuss later, with an estimated error of about $\pm 1^{\circ}\text{C}$ (supporting materials).

There is a striking similarity between our deep-water temperature record and the 800-ky record of atmospheric temperature above Ant-

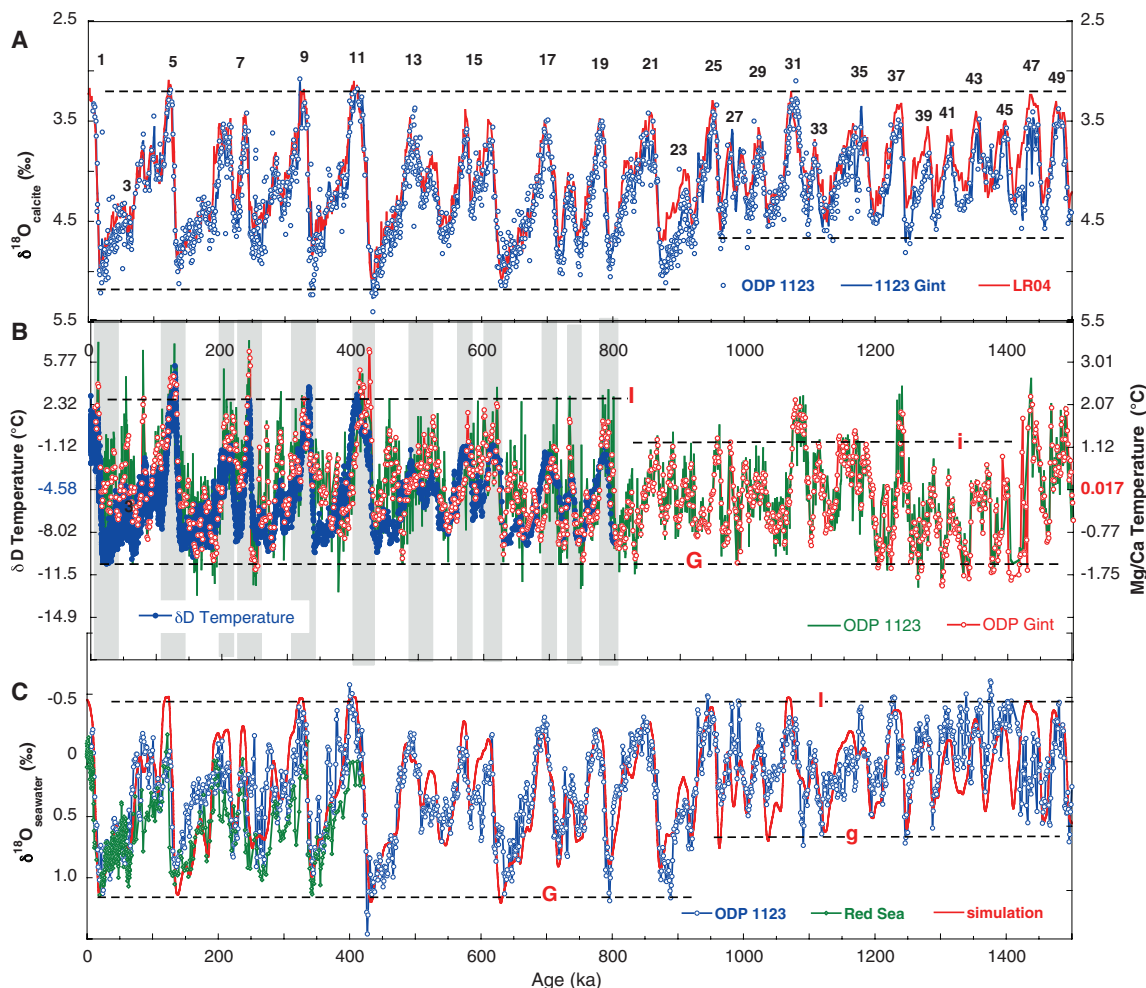
arctica from ice cores (16) (Fig. 1B). For this comparison, we converted Mg/Ca temperature data to units of standard deviation and transposed them to the EDC3 (Epica Dome C ice core) time scale (20). The pattern seen in the ice-core δD (deuterium isotope-based temperature) record is matched by the Mg/Ca-based deep-sea temperature: (i) glacial temperatures are similar throughout this deep-sea record, (ii) interglacial temperatures are cooler prior to 450 ka, and (iii) interglacials 5.5, 7.5, 9.3, and 11.3 are exceptionally warmer.

The distinction between interglacial bottom-water temperature before and after ~450 ka seen in our record and in the ice-core atmospheric record is also similar to a sub-Antarctic Atlantic sector record of sea surface temperature using an alkenone proxy (21) and of the oxygen isotopes of that site (22). It is also recognized in model-based deconvolution of global deep-sea temperature from the benthic $\delta^{18}\text{O}$ stack (11). However, it is not seen in the North Atlantic benthic Mg/Ca record referred to earlier (6). It is unclear whether this reflects the linkage of that site to hydrographic conditions associated with changing proportions of NADW and AABW or is somehow a mixed signal related to a contribution from carbonate ion as discussed earlier (9, 10).

Glacial-interglacial model comparisons (23) suggest that Antarctic temperature changes represent global-scale temperature changes with a polar amplification factor of ~2: glacial-interglacial temperature change (Antarctic/Global) = 2.07. The comparison of Antarctic and our deep-sea temperature data shows the same amplification (Antarctic/deep sea) = 2.1 ± 0.08 (fig. S5). Therefore, it seems that the temperature of the bottom water reflects the air temperature at southern high latitudes and thus Southern Ocean surface temperatures, aided by upwelling and surface mixing of CDW.

Ice volume and sea level. Given Mg/Ca, rearrangement of the paleotemperature equation allows changes in the $\delta^{18}\text{O}_{\text{W}}$ ($\Delta\delta^{18}\text{O}_{\text{W}}$) to be calculated [(15) and supplementary text]. We made two tests of the methodology. First, we compared our results over the past 250 ky with estimates of global sea level made from accurately dated corals. The coral method is independent of orbital-based $\delta^{18}\text{O}_{\text{C}}$, whereas other sea-level methods rely on comparison with benthic $\delta^{18}\text{O}$. Considering that propagation of estimated temperature and $\delta^{18}\text{O}_{\text{C}}$ uncertainties results in an error in $\delta^{18}\text{O}_{\text{W}}$ of ± 0.2 ‰, our results (fig. S6) show excellent agreement with compilations of coral data (24, 25). Second, it can be seen that

Fig. 1. Records on the LR04 chronology of (A) The LR04 benthic $\delta^{18}\text{O}$ stack (19) (red); ODP 1123 benthic $\delta^{18}\text{O}$ (original data in blue open symbol and smoothed record with a Gaussian filter having a span of 5 ky as a line in blue); (B) Record on the EDC3 chronology (20) of ODP 1123 benthic Mg/Ca temperature (original data in green and smoothed record with a 5-ky Gaussian filter red) and Antarctic temperature (16) (blue). Data were converted to units of standard deviation. Ordinate axes show mean temperatures (in red and blue) and also values of mean $\pm 3\sigma^{\circ}\text{C}$. MIS is labeled for orientation in Fig. 1A and in shaded panels in Fig. 1B. (C) Ice volume ($\delta^{18}\text{O}$ of seawater) derived from ODP 1123 Mg/Ca and $\delta^{18}\text{O}_{\text{C}}$ (blue) compared with the simulation of Bintanja *et al.* (11) (red) and record from the Red Sea (28) (green).



the change in $\delta^{18}\text{O}_\text{W}$ since the Last Glacial Maximum is $\sim 1.2\text{‰}$ for 120 m of sea-level change, identical to the value of $1.1 \pm 0.1\text{‰}$ derived from porewater modeling at this Chatham Rise site and similar to global estimates (26). This exercise goes some way toward addressing the issue discussed earlier on the influence of local hydrography on the site 1123 $\delta^{18}\text{O}_\text{C}$ record.

One further factor in estimating sea level by this approach is the simplifying assumption of a constant $\delta^{18}\text{O}_\text{W}$ through a glacial cycle, whereas $\delta^{18}\text{O}$ of ice ($\delta^{18}\text{O}_\text{I}$), and $\delta^{18}\text{O}_\text{W}$, will increase as the ocean is progressively depleted of ^{16}O (27). Assumption of $\delta^{18}\text{O}_\text{W}$ based on a constant $\delta^{18}\text{O}$ of ice has little impact on derivation of sea level estimated from $\delta^{18}\text{O}_\text{W}$ records during the start of a glacial cycle but has a larger impact at full glaciation (fig. S7). Here, we have used $\delta^{18}\text{O}_\text{I} = -40\text{‰}$. On this basis, we have calculated the $\delta^{18}\text{O}_\text{W}$ and, assuming that this reflects global ice volume, derived a sea-level reconstruction since 1550 ka and compared it with a recent simulation and record of sea-level change (11, 28) (Fig. 1C).

First, we estimate the contributions of $\delta^{18}\text{O}_\text{W}$ and temperature to $\delta^{18}\text{O}_\text{C}$, a subject of long-standing debate (1–4). Temperature has been scaled as $\delta^{18}\text{O}_\text{T}$, and all data are plotted as “cold upwards” to show the evolution of temperature and ice volume from interglacial to glacial conditions (Fig. 2, A and B). We see different behavior between 100,000-year (Fig. 2, A and B) and 41,000-year (Fig. 2, C and D) cycles, periodicities consistent with the tempo of variations in orbital eccentricity and obliquity. This exercise shows that the increase in $\delta^{18}\text{O}_\text{W}$ (ice volume) during eccentricity cycles proceeds slowly throughout some glacial periods to a maximum value that corresponds to a $\sim 70\%$ contribution to $\delta^{18}\text{O}_\text{C}$, but there are also periods of rapid change. In contrast, $\delta^{18}\text{O}_\text{T}$ cools early in each glacial cycle by about 0.8‰ . The contrast between the rapid temperature decrease completed one-third of the way through each $\delta^{18}\text{O}_\text{C}$ cycle and the more gradual lagged increase in ice volume has two implications. One is that, as suggested by the simulation of Bintanja *et al.* (11), rapid deep-ocean cooling preceded slower buildup of ice sheets. The other is that the near constancy of glacial $\delta^{18}\text{O}_\text{T}$ (red dashed line in Fig. 2, A and B), irrespective of the strength of individual $\delta^{18}\text{O}_\text{C}$ maxima, suggests that glacial cooling is limited by water’s freezing temperature such that glacial temperatures have remained nearly constant since at least 1500 ka. This analysis also shows that the saw-tooth character of the oxygen isotope cycles is a feature of the $\delta^{18}\text{O}_\text{W}$ component of the record—slow ice-sheet buildup and rapid decay—whereas the temperature component resembles a square wave function resulting from the limitation of the temperature fall during the early stages of ice-sheet growth.

The records over obliquity cycles before about 1 million years ago show a much weaker role for ice volume because of smaller ice sheets.

The $\Delta\delta^{18}\text{O}_\text{W}$ component of the record is $0.77 \pm 0.16\text{‰}$ for MIS 24 to 50, compared with $1.09 \pm 0.17\text{‰}$ over MIS 2 to 22 (fig. S8), equivalent to a sea-level fall from ~ 60 to 90 m for the obliquity cycles to ~ 90 to 125 m for the eccentricity cycles. However, $\Delta\delta^{18}\text{O}_\text{T}$ shows no such contrast and averages $0.80 \pm 0.14\text{‰}$ over all glacials sampled, equivalent to cooling of about 3°C to -2°C (Fig. 2C).

This study emphasizes the point that benthic $\delta^{18}\text{O}$ is not a direct proxy of ice volume or sea level (1–3). Because the LR04 stack (19) is often taken as a proxy for ice volume, it is interesting that the temperature component of this record may approach as much as 50% of the range in $\delta^{18}\text{O}_\text{C}$.

Timing of MPT events. With this assessment of the relative contributions of temperature and ice volume to $\delta^{18}\text{O}_\text{C}$, it is possible to address what was the sequence of events leading to the MPT. A review of $\delta^{18}\text{O}_\text{C}$ records (5) has shown that the MPT began 1250 ka and was complete

by 700 ka, with low-frequency (i.e., 100 ky) power emerging at about 1250 ka followed by a lull centered on 1000 ka, with reemergence of power beginning at ~ 900 ka. Wavelet analysis of our $\delta^{18}\text{O}_\text{C}$, $\delta^{18}\text{O}_\text{W}$, and Mg/Ca records using Sowas software (29, 30) shows somewhat similar but more complex patterns (Fig. 3). In the $\delta^{18}\text{O}_\text{W}$ record, the highest spectral power in the 100-ky band develops from 650 ka. Temperature gains power in this band over 400 to 100 ka, whereas $\delta^{18}\text{O}_\text{C}$ is a “fusion” of the two with lower yet significant power from 900 ka and intensified power from ~ 650 ka.

The record of glacial deep-ocean temperatures over this period is similar to that which characterizes glacials at this site throughout the Pleistocene. As shown above, rapid deep-ocean cooling within each glacial-interglacial cycle preceded slower buildup of ice sheets, but there is no evidence of progressive cooling throughout the record; glacial to interglacial temperature change ($\Delta\delta\text{T}$)

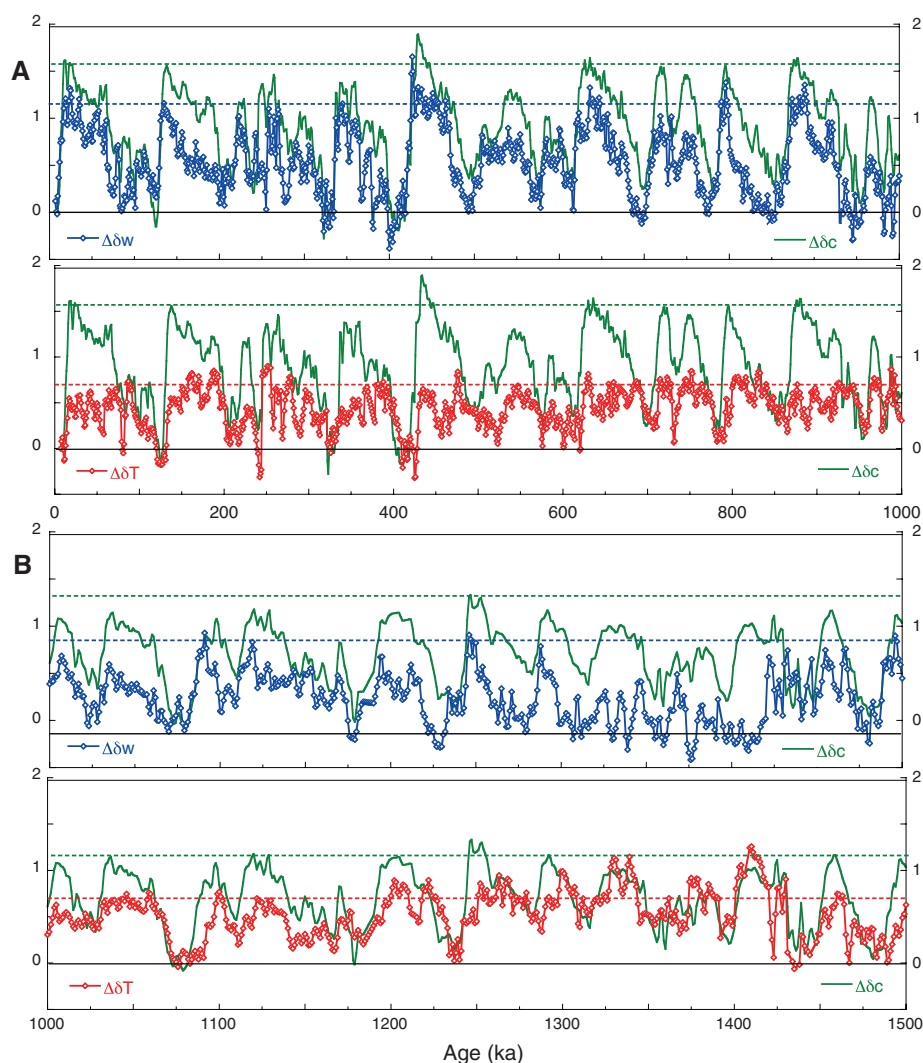


Fig. 2. Comparison of contributions of changes in ice volume, $\Delta\delta\text{W}$ (blue) and temperature, ΔT (red) to benthic $\delta^{18}\text{O}$, $\Delta\delta\text{C}$ (green): (A) for 0 to 1000 ka and (B) for 1000 to 1500 ka. Data are plotted as “cold upwards.” Temperature converted to δT ‰ units using 0.25‰ per $^\circ\text{C}$ /0.1 mmol/mol per $^\circ\text{C}$. Histogram of data shown in fig. S8.

is constant at about 0.8‰ over every glacial cycle except for the warm interglacials. Temperature decline cannot therefore be the unique cause of increased ice volume across the MPT. However, a characteristic of the eccentricity cycles, less so for obliquity, is the persistence of near-freezing temperatures over most of the periods of ice growth; the longer slow growth of ice sheets occurred when cold temperatures persisted for longer.

In contrast to temperature, the change in ice volume is late within the MPT period, and its timing coincides with the so-called 900-ka event at the onset of MIS 22 (Figs. 1C and 4A). Before this event, the record oscillates between mild glacials (g) and lukewarm interglacials (i), suggesting that the climate system varied between two quasistable climate states (31, 32). After MIS 25, ice volume increased sufficiently to weaken interglacial MIS 23 and initiated the onset of full glacial conditions (G) beginning with MIS 22 (~900 ka). MIS 22 marks an abrupt increase in glacial $\delta^{18}\text{O}_\text{W}$ values, indicating a transition to maximum ice volume that has characterized most full glacial (G) stages since ~900 ka. Our results suggest that the MPT represents an abrupt reorganization of the climate system as opposed to a long-term trend toward increased ice volume and colder temperatures as previously thought (5). The duration of the glacial cycle lengthens with MIS 22 to 24, but the strong increase in the 100-ky power is delayed until ~650 ka (MIS 16), which is where power in $\delta^{18}\text{O}_\text{W}$ reaches its maximum (Fig. 3). From 900 to 400 ka, the cli-

mate system varied between three end-member climate states: lukewarm interglacials (i), mild glacials (g), and full glacials (G) [after Paillard (31)]. It is not until 400 ka during the mid-Brunhes event that full interglacial conditions (I) emerged, most vividly expressed in the deep-sea temperature record.

Although suggestions that the MPT was a gradual transition are shown in a number of benthic $\delta^{18}\text{O}$ records, our evidence for a more abrupt transition comes from seawater $\delta^{18}\text{O}_\text{W}$ (Fig. 1C). We attribute this difference to hydrographic contributions to $\delta^{18}\text{O}_\text{C}$.

What conditions may have led to the abrupt increase in continental ice volume during MIS 22? Examination of the sea-level (ice volume) record across the MPT (Fig. 4A) shows that the critical step in ice-volume variation was associated with the suppression of melting in MIS 23, followed by renewed ice growth in MIS 22 to yield a very large ice sheet with 120 m of sea-level lowering (Fig. 4A). This period is associated with an anomalously low Southern Hemisphere summer insolation across the minor melting MIS 23. We suggest that this configuration suppressed melting and allowed larger ice-sheet growth that led to the first prolonged 100-ky glacial cycle comprising MIS 22 to 24. Because the low summer insolation occurred in the Southern Hemisphere, we suggest that the cause of ice volume increase at 900 ka lay in Antarctica.

Cross-spectral analysis of our records shows that at eccentricity periods, $\delta^{18}\text{O}_\text{C}$ lags Mg/Ca (temperature) by ~12 ky, as found earlier (15, 33),

and $\delta^{18}\text{O}_\text{W}$ lags Mg/Ca by ~21 ky with, as expected, $\delta^{18}\text{O}_\text{C}$ intermediate between $\delta^{18}\text{O}_\text{W}$ and temperature; see supplementary text and fig. S9 for discussion of the phase relationship between orbital eccentricity and Mg/Ca (temperature). The 41-ky bandpass filtered data show, as in (33), in-phase relationships between all measured parameters, whereas the 100-ky bandpass filtered data shows considerable variability in phase (fig. S9). Over the period from ~1100 to 750 ka, where 100-ky power is weak, the lag increases to the extent that $\delta^{18}\text{O}_\text{C}$ and $\delta^{18}\text{O}_\text{W}$ are out of phase with δT at 900 ka, after which there is a transition to strong 100-ky cycles from ~700 ka. Thus, the “lock in” to a late Pleistocene 100-ky phase relationship occurs at around 750 ka, with some variation before then.

The notion that the Antarctic ice sheet was inactive throughout the Pleistocene is not supported by our work or by records emanating from the margins of Antarctica and some models (34–37). Raymo *et al.* have proposed a more active East Antarctic ice sheet before 1 Ma with its expansion and development of the present less-active regime at the MPT (34). Their proposal—that at the MPT, “marine-based ice-sheet margins replaced terrestrial ice margins around East Antarctica, resulting in a shift to in-phase behavior of northern and southern ice sheets” (34)—is borne out by drill cores on the edge of the Ross Sea (35, 37). Pollard and De Conto (36) model an increase in West Antarctic Ice Sheet ice volume of ~10⁷ km³ (~17 m of sea level) across the MIS 25 to 22 transition.

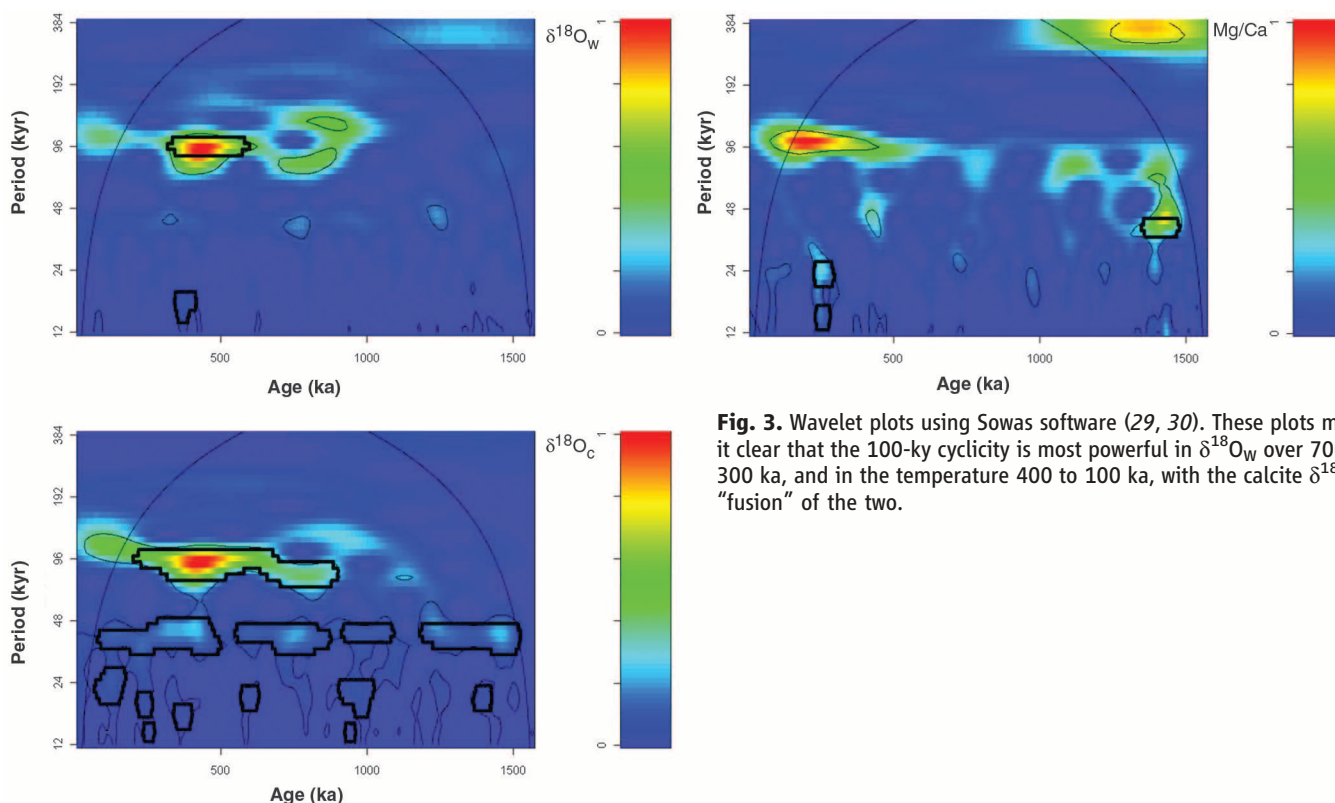


Fig. 3. Wavelet plots using Sowas software (29, 30). These plots make it clear that the 100-ky cyclicality is most powerful in $\delta^{18}\text{O}_\text{W}$ over 700 to 300 ka, and in the temperature 400 to 100 ka, with the calcite $\delta^{18}\text{O}$ a “fusion” of the two.

Carbon chemistry and ocean circulation. The rapid increase in ice volume at MIS 22 is associated with a large perturbation to ocean carbon chemistry (38–40), as reflected in the most depleted benthic $\delta^{13}\text{C}$ values in the Pacific and Atlantic over the past 5 million years (36–38). This is also seen in the ODP 1123 record (Fig. 4A and Fig. 5). Because it is represented in many records, some authors have argued that this decrease in oceanic $\delta^{13}\text{C}$ is a global event. It has also been argued that benthic $\delta^{13}\text{C}$ values begin to decline much earlier than at MIS 22, at about 1.5 Ma (41–43), reaching a minimum at 900 ka and increasing to about 500 ka. However, our results suggest that the carbon isotope excursion was associated with an abrupt reorganization of the climate system rather than a long-term trend, characterized by the most depleted $\delta^{13}\text{C}$ values at the MPT within a record of otherwise rather similar glacial values (Fig. 5). Therefore we will focus first on the extreme depletion in $\delta^{13}\text{C}$ at the MPT and next consider its glacial-interglacial cycles.

We can dismiss the hypothesis that the $\delta^{13}\text{C}$ record for this site, which is based on an infaunal foraminiferal species, is depleted relative to bottom water and reflects productivity-related changes in pore-water composition. This is because paired *Cibicides-Uvigerina* data (fig. S10) show a constant offset of $\sim 0.9\text{‰}$ between the epifaunal and infaunal species. The *Uvigerina* spp. $\delta^{13}\text{C}$ record cannot therefore reflect changes in metabolic CO_2 at the depth of this ODP site. Given this, we can explore interpretations of the benthic $\delta^{13}\text{C}$ record, first exploiting the ice-volume record that we have derived.

Before 900 ka, sea-level lowstand during glaciations was ~ 70 m below the present. MIS 22 represents the first time in millions of years that sea level dropped to 120 m, exposing shelf break and upper-slope deposits (38). Marine organic carbon (with $\delta^{13}\text{C}$ of $\sim -18\text{‰}$) exposed on shelves and transferred to seawater would lower benthic $\delta^{13}\text{C}$. The depletion in $\delta^{13}\text{C}$ compared with “normal” glaciials (for example, MIS 20, 24, and 26) is about 0.2‰ , equivalent to about 300 Pg of carbon. A test of this hypothesis is to examine the relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}_{\text{W}}$. Ice volume and $\delta^{13}\text{C}$ look very tightly coupled in Fig. 4A and Fig. 5. This strong link between $\delta^{18}\text{O}_{\text{W}}$ and $\delta^{13}\text{C}$ suggests that sea level and/or circulation is involved in glacial-interglacial $\delta^{13}\text{C}$ changes. This is a more likely cause than change in the terrestrial carbon reservoir resulting from global aridity, as originally conceived by Shackleton (44). The 41-ky bandpass filtered data show (as above for other climate variables) that $\delta^{18}\text{O}_{\text{W}}$ and $\delta^{13}\text{C}$ are closely in phase (fig. S9) but the responses in the 100-ky band after 900 ka show a small lead of $\delta^{13}\text{C}$ to $\delta^{18}\text{O}_{\text{W}}$. Taken at face value, this does not support the shelf hypothesis over 100-ky time scales.

The second interpretation, argued indirectly from several avenues [summarized in (45)], is that benthic $\delta^{13}\text{C}$ is controlled by the hydro-

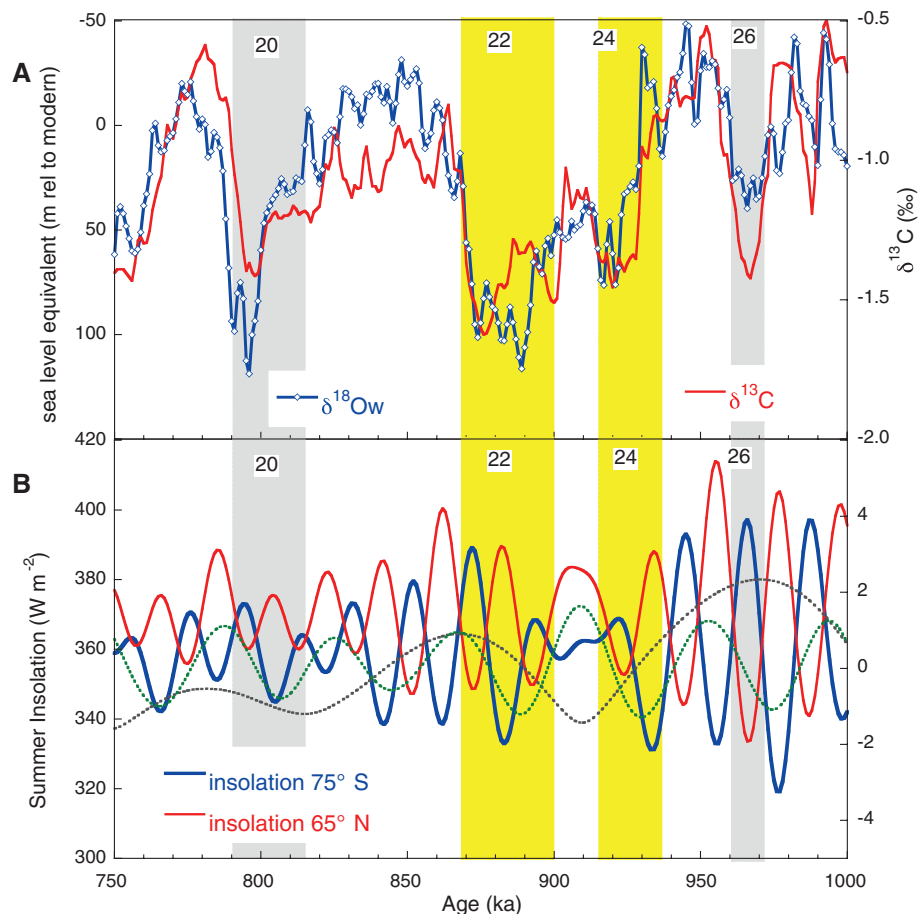


Fig. 4. (A) Record over 750 to 1000 ka showing age of the MPT at MIS 22 inferred from $\delta^{18}\text{O}_{\text{W}}$ (sea level) (blue). Also shown is benthic $\delta^{13}\text{C}$ (red). MIS with boundaries taken from LR04 (19) are labeled and shown in shaded panels for orientation. (B) Normalized orbital variation in energy received by Earth at eccentricity and obliquity (blue and green dashed lines) and Northern and Southern Hemisphere (red and blue lines) insolation frequencies for the period 1 Ma to 800 ka; note the antiphase relation between obliquity and a wide Southern Hemisphere insolation peak at 920 to 900 ka.

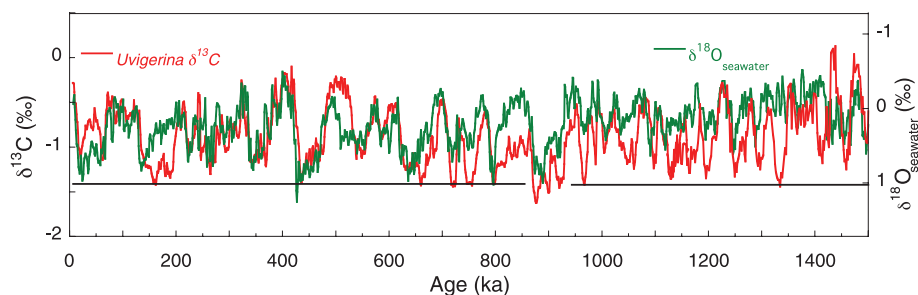


Fig. 5. Record of benthic $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of seawater. $\delta^{13}\text{C}$ at MIS 22 is most depleted of benthic $\delta^{13}\text{C}$ values in the Pacific and Atlantic over the past 5 million years (42).

graphic properties of site ODP 1123 (mostly in addition to fluctuations in the marine C reservoir) affected by changes in global thermohaline circulation with or without concomitant change in nutrients (44, 45). As discussed above, differences between $\delta^{18}\text{O}_{\text{C}}$ records of site 1123 and the LR04 stack provide some evidence of a secondary hydrographic control. We have tested this hypothesis using Nd isotopes that act as a

hydrographic tracer largely unaffected by changes to organic carbon systematics. Northern source waters have a more negative ϵ_{Nd} than southern source waters (46). If the $\delta^{13}\text{C}$ changes reflect changes in proportions of water masses, we would anticipate that they should be associated with concomitant changes in ϵ_{Nd} . We examined three periods: over the last deglaciation, the warm interglacial 100-ky cycles MIS 12 to 10, and

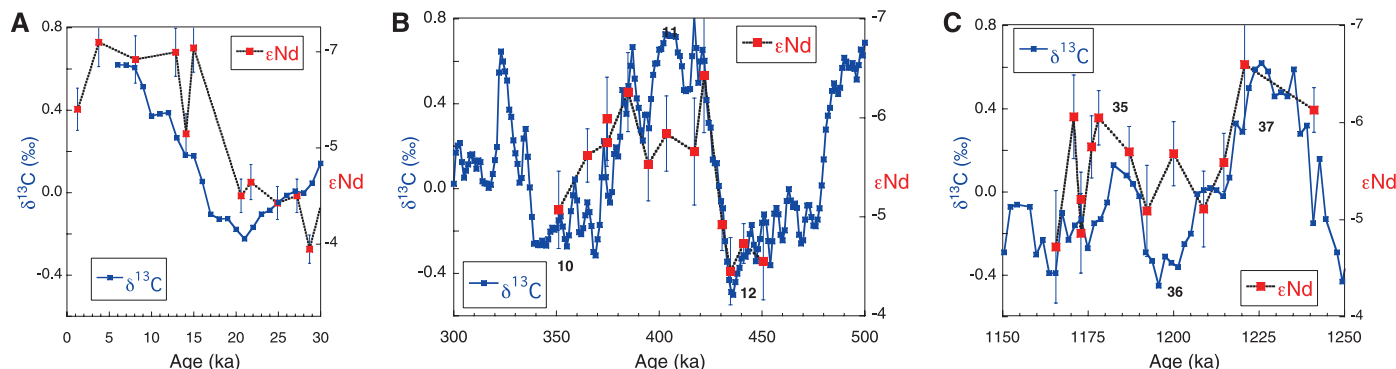


Fig. 6. Records of ϵNd (red) and $\delta^{13}\text{C}$ (blue) for three periods within core ODP 1123: (A) 0 to 30 ka, (B) 300 to 500 ka, (C) 1150 to 1250 ka. All show that in warm periods, ϵNd is more negative, suggesting greater NADW influence.

the obliquity cycles MIS 38 to 35 (Fig. 6). In each case, the records of ϵNd and $\delta^{13}\text{C}$ are similar in form, with a small range in ϵNd from about -7 at interglacial stages to -4 at glacial stages. This implies that the 100-ky and 40-ky cycles of $\delta^{13}\text{C}$ are associated with changes in deep-ocean circulation, perhaps a decrease in glacial export of NADW relative to CDW coupled with changes in Southern Ocean deep-water ventilation (17, 33, 39). However, given the location of ODP 1123, there is little scope for large variations in end members.

Plots of the relationship between ϵNd and $\delta^{13}\text{C}$ data reveal that large changes in the oceanic carbon reservoir occur in addition to those in $\delta^{13}\text{C}$ that are associated with changes in ϵNd (fig. S11). Of the glacial-interglacial offset in $\delta^{13}\text{C}$ of about 1‰ (Fig. 5), about half is associated with circulation and half with carbon reservoir. A whole-ocean decrease in $\delta^{13}\text{C}$ of 0.4‰ would represent about half of the $\delta^{13}\text{C}$ change over glacial-interglacial cycles and would require transfer of about 500 Pg of terrestrial carbon to the oceans, together with an increase in atmospheric CO_2 of 45 parts per million by volume before compensation.

Importance. Most hypotheses account for the origin of the MPT as a response to long-term ocean cooling, perhaps because of lowering CO_2 (5). Data of CO_2 , directly from ice cores and indirectly from the $\delta^{11}\text{B}$ proxy (47) (fig. S12), are as yet too sparse to determine the respective roles of temperature and of the carbon system. We have defined the timing of initiation of the MPT as an abrupt event centered on MIS 24 to 22 (the 900-ka event). The descent into longer glacials, the MPT, appears to have begun in MIS 23 by suppression of substantial melting of the ice formed in MIS 24, perhaps due to seasonality effects originating in the Southern Hemisphere, before the renewed ice growth in MIS 22 yielded a very large ice sheet. We see no evidence of a pattern of cooling since 1500 ka, but near-freezing temperatures occur at every glacial maximum. The records over obliquity cycles before about 1 million years ago show a much weaker role for ice volume because of smaller ice sheets.

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Divergent Nematic Susceptibility in an Iron Arsenide Superconductor

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Within the Landau paradigm of continuous phase transitions, ordered states of matter are characterized by a broken symmetry. Although the broken symmetry is usually evident, determining the driving force behind the phase transition can be complicated by coupling between distinct order parameters. We show how measurement of the divergent nematic susceptibility of the iron pnictide superconductor $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ distinguishes an electronic nematic phase transition from a simple ferroelastic distortion. These measurements also indicate an electronic nematic quantum phase transition near the composition with optimal superconducting transition temperature.

In an electronic nematic phase transition, the electronic system breaks a discrete rotational symmetry of the crystal lattice without altering the existing translational symmetry (*I*). Examples include half-filling quantum Hall states

(2) and the field-induced metamagnetic state in $\text{Sr}_3\text{Ru}_2\text{O}_7$ (3). In the latter case, the electronic ground state exhibits a strong twofold anisotropy, which is only weakly reflected in a subtle structural anisotropy (4) indicative of an electronically driven phase transition. Recently, both cuprates (5–8) and iron pnictides (9–12) have been proposed as candidates for an electronic nematic phase, in which nematic order might coexist with high temperature superconductivity. However, the argument for electronic nematicity in these compounds is not straightforward because the crystal lattices suffer a relatively large deviation from fourfold symmetry. We report measurements of

the resistivity anisotropy of $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ induced by an in situ tunable strain, which reveals the intrinsic electronic nematic response under a constant lattice distortion. On the basis of a phenomenological Ginzburg-Landau model, we show that the structural phase transition in this representative iron pnictide superconductor is purely driven by an instability in the electronic part of the free energy.

We apply a tuneable in-plane uniaxial strain to single crystal samples of $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ to probe the nematic response. By gluing the sample on the side wall of a piezo stack (Fig. 1A), strains can be applied by the deformation of the piezo, which is controlled by an applied voltage (V_p) (13, 14). The strain (i.e., the fractional change of length along the current direction, $\epsilon_p = \Delta L/L$) was monitored via a strain gauge glued on the back side of the piezo stack. Both ϵ_p and the fractional change of resistivity ($\eta = \Delta\rho/\rho_0$, where ρ_0 is the resistivity of the free-standing sample before gluing on the piezo stack) were measured at constant temperature while the applied voltage was swept (Fig. 1B). The voltage dependence of η and ϵ_p shows hysteretic behavior because of the ferroelectric nature of the piezo materials, yet the two quantities exhibit a linear relationship without any hysteresis (Fig. 1B). The negative slope of $\eta(\epsilon_p)$ indicates that the resistivity is higher along the shorter bonding direction, consistent with previous results (10, 15, 16).

The amount of strain transmitted to the sample (ϵ_s) can be assessed by gluing another strain

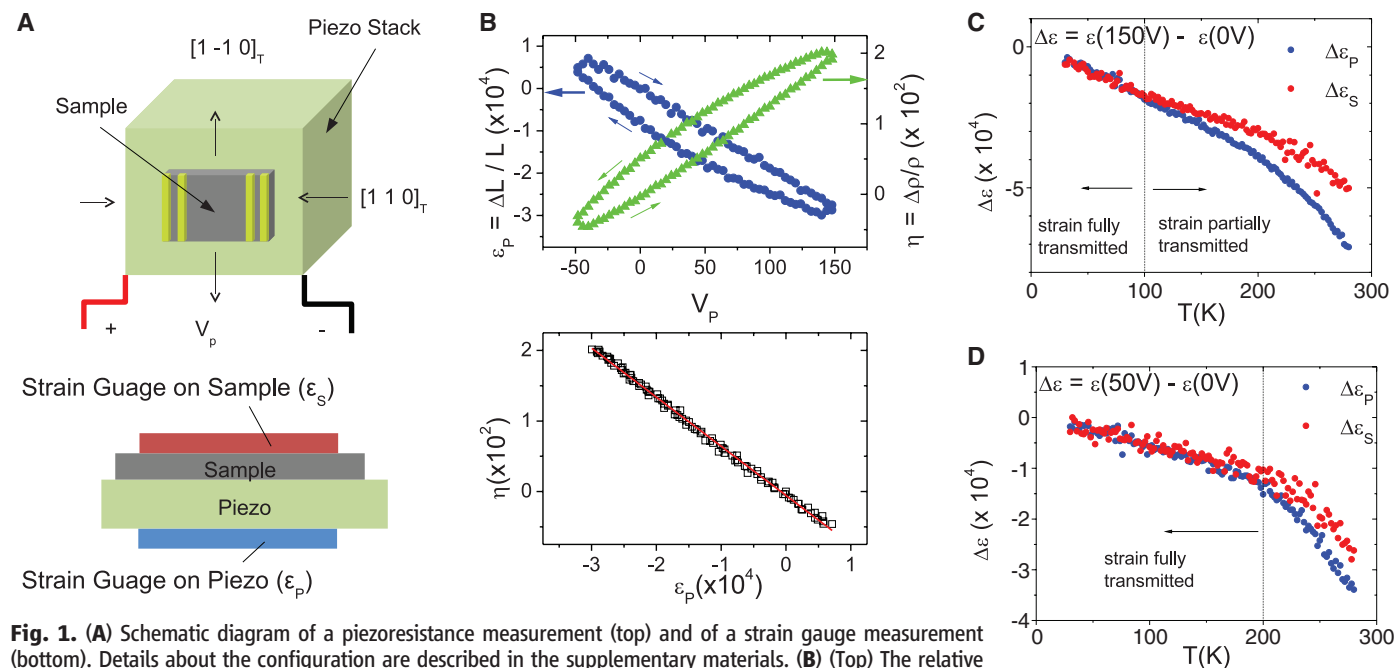


Fig. 1. (A) Schematic diagram of a piezoresistance measurement (top) and of a strain gauge measurement (bottom). Details about the configuration are described in the supplementary materials. (B) (Top) The relative change in resistivity ($\eta = \Delta\rho/\rho_0$) of a BaFe_2As_2 sample and the strain measured by a strain gauge on a piezo ($\epsilon_p = \Delta L/L$) as a function of voltage at $T = 140$ K. The strain and resistance were measured along the $[1\ 1\ 0]_T$ direction of the crystal. (Bottom) Same set of data but η is plotted against ϵ_p . The red line is a linear fit to the data. (C and D) The differences in the strain between zero applied voltage and (C) $V_p = 150$ V and (D) $V_p = 50$ V. Vertical lines indicate the temperatures below which the strain is fully transmitted to the sample. For low voltage, this temperature window spans well above T_s for all compositions studied.

gauge on the top surface of the crystal (Fig. 1A, lower panel). The comparison of ϵ_s and ϵ_p for a $\text{Ba}(\text{Fe}_{0.955}\text{Co}_{0.045})_2\text{As}_2$ sample is summarized in Fig. 1, C and D. For applied voltages $|V_p| < 150$ V, the strain is fully transmitted to the sample for temperatures below about 100 K for typical thickness crystals (less than 100 μm). For lower voltages, $|V_p| < 50$ V, the strain is fully transmitted to even higher temperatures (Fig. 1D). The maximum strain that can be applied ($|\epsilon| < 5 \times 10^{-4}$) is substantially less than the lattice distortion developed below the phase transition ($10^{-2} \sim 10^{-3}$), and, as we show below, the system is always in the regime of linear response.

The induced fractional change of the resistivity η provides a direct measure of the electronic nematic order parameter. In general, the resistivity is determined by both the electronic structure and the scattering. In the case of iron pnictides, the proposed orbital ordering more likely results in an anisotropy of electronic structure, whereas the spin-nematic ordering leads to an anisotropy of electron scattering. Measurements of resistivity anisotropy $\psi = (\rho_b - \rho_a)/(\rho_b + \rho_a)$ pick up both effects and reveal the electronic nematic order (17–19). For a strained crystal in the tetragonal state, ρ_b and ρ_a refer to the resistivity in directions parallel and perpendicular to the applied compressive stress. It can be easily shown that $\eta = \psi$ if the increase in ρ_b equals the decrease in ρ_a and that the two quantities are directly proportional even if this is not the case. The same is also true for the derivatives of these quantities such that $d\eta/d\epsilon \propto d\psi/d\epsilon$ (13).

Representative data showing the electronic nematicity (η) as a function of strain (ϵ_p) for a BaFe_2As_2 sample are shown in Fig. 2A at various temperatures above the structural transition temperature T_s . Data were fit by a straight line in a small range of strain near zero applied voltage. As shown in Fig. 2B, the quantity $d\eta/d\epsilon$, which essentially measures the nematic response induced by a constant strain, diverges upon approaching T_s from above. This divergent behavior is reminiscent of the resistivity anisotropy ob-

served above T_s for samples held in a mechanical clamp (10). However, as we explain below, there is an important distinction between measurements made under condition of constant stress (mechanical clamp) and constant strain (measurement of $d\eta/d\epsilon$ in the current set up).

From the thermodynamic point of view, the stress and strain are conjugate variables, and the stress (here denoted as h) is the externally controllable force, whereas strain is the response of a mechanical system. Intuitively it might be more reasonable to regard stress as a symmetry breaking field. However, from the electron nematic stand point, stress only couples indirectly to the nematic order parameter through strain. This relationship can be best understood from the following Ginzburg-Landau free energy:

$$F = \frac{a}{2}\psi^2 + \frac{b}{4}\psi^4 + \frac{c}{2}\epsilon^2 + \frac{d}{4}\epsilon^4 - \lambda\psi\epsilon - h\epsilon \quad (1)$$

Here, ψ represents the electronic nematic order parameter, measured by the resistivity as discussed above, ϵ is the elastic strain, and h is its conjugate stress. a , b , c , and d are the coefficients of the two order parameters in the usual power series expansion, and λ is the coupling constant. If there is a phase transition driven by the electronic degree of freedom, then the coefficient a becomes zero at some temperature, that is, $a = a_0(T - T^*)$, whereas the other coefficients are temperature independent. On the other hand, if the phase transition is caused by a structural instability, then it is the coefficient c that becomes zero [$c = c_0(T - T^*)$] (20). Therefore, the driving force can be distinguished by determining the temperature dependence of the bare a and c coefficients.

With this in mind, we can now ask what the difference is between measuring the response of electronic nematicity ψ under constant strain ϵ rather than constant stress h . This can be answered explicitly by calculating the quantities $d\psi/dh$ and $d\psi/d\epsilon$ under the constraint of minimizing the free energy (13):

$$\frac{d\psi}{dh} = \frac{\lambda}{ac - \lambda^2} \quad (2)$$

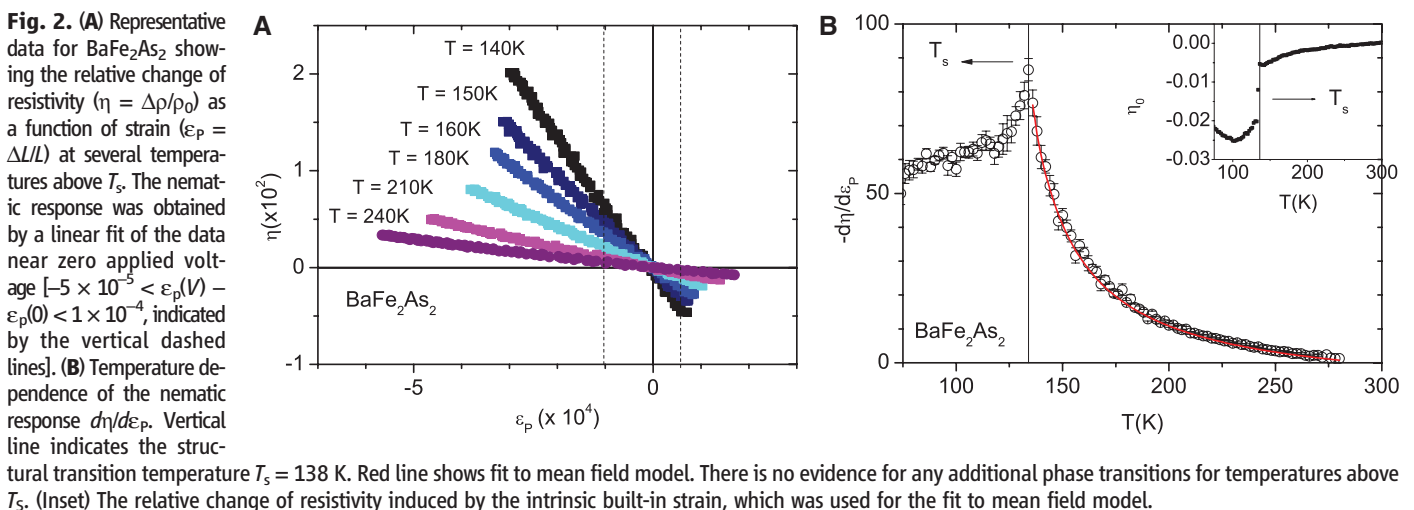
$$\frac{d\psi}{d\epsilon} = \frac{\lambda}{a} \quad (3)$$

From these expressions, the nematic response under a constant stress (Eq. 2) will show a $1/T$ divergence whether the driving force is a structural or electronic phase transition. However, the nematic response under a constant strain will only diverge when it is a true electronic nematic phase transition (Eq. 3). In this sense, the divergence in $d\eta/d\epsilon$ shown in Fig. 2 is direct evidence that BaFe_2As_2 suffers a true electronic nematic instability, and the structural transition merely passively follows the nematic order. Because strain is a field to the nematic order parameter, we refer to the quantity $d\psi/d\epsilon$ as the nematic susceptibility.

From Eq. 3, $d\psi/d\epsilon = \lambda/a = \lambda/[a_0(T - T^*)]$, it is natural to fit the data of $d\eta/d\epsilon$ in Fig. 2B with a Curie-Weiss temperature dependence. However, Eq. 3 is only valid in the limit of vanishing strain, at which one can disregard the higher-order nonlinear terms. In the realistic experiment situation, there is always some intrinsic built-in strain, even at zero applied voltage, because of the different thermal contraction of the sample and the piezo stack. To take into account this built-in-strain, we perform a numerical fit based on the following expression:

$$\frac{d\eta}{d\epsilon} = \frac{\lambda}{a_0(T - T^*) + 3b\eta_0^2} + \chi_0 \quad (4)$$

The effect of the next-order nonlinear term is included in the $3b\eta_0^2$ in the denominator, where η_0 is the resistivity anisotropy induced by the built-in strain as a function of temperature, measured by the difference of resistivity of a sample before and after gluing on the piezo stack. In addition to a_0 and b introduced before, χ_0 is a fitting parameter to model the intrinsic piezoresistivity effect of the



materials that is unrelated to the electronic nematic phase transition.

The result of this fitting is plotted in Fig. 2B as a solid red curve, which is in excellent agreement with measured data $d\eta/d\varepsilon$. The mean field critical temperature T^* obtained from the fitting is 116 K, 22 K lower than the actual phase transition temperature ($T_s = 138$ K). This can also be understood from the Ginzburg-Landau free energy in Eq. 1. By minimizing the free energy, we can derive the result that the nonzero nematic and structural order parameters onset simultaneously at a temperature $T_s = T^* + \lambda^2/(a_0c)$, higher than T^* (13). This is a consequence of the bilinear coupling between the electronic nematic system and the crystal lattice, which lifts the critical temperature of the electronic instability to a higher temperature. Physically, the lattice provides a polarizable medium, which enhances the nematic instability.

The divergence of $d\eta/d\varepsilon$ not only reveals the tendency toward an electronic nematic phase

transition but also measures the strength of nematic fluctuations, according to the fluctuation-dissipation theorem. We have measured $d\eta/d\varepsilon$ of $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ samples for doping concentration ranging from the undoped parent compound to overdoped compositions (Fig. 3). The magnitude of $d\eta/d\varepsilon$ is plotted as a color map in the composition versus temperature phase diagram in Fig. 4. For the underdoped part of the phase diagram, $d\eta/d\varepsilon$ increases rapidly near the structural phase transition boundary. As the doping concentration increases, the intensity of fluctuations increases and reaches a maximum near optimal doping concentration, where structural and magnetic transitions are fully suppressed. The nematic fluctuations persist to the overdoped regime, eventually decreasing as the superconducting T_c decreases. The associated softening of the shear modulus has been extensively studied by resonant ultrasound measurements (11, 21).

To quantitatively track the evolution of nematic fluctuations across the phase diagram, we performed numerical fits to the data for each composition based on Eq. 4. The obtained T^* is also plotted as a function of composition in Fig. 4. The mean field nematic critical temperature T^* closely tracks the actual structural transition temperature T_s in the underdoped regime and is suppressed to zero at the optimal doping. T^* becomes negative as the doping further increases beyond optimal doping, indicating a “paranematic” state. Our experimental data and analysis indicate an electronic nematic quantum phase transition for a composition close to optimal doping (22). It remains to be seen whether fluctuations associated with this quantum phase transition play an important role in enhancing T_c in the superconducting phase. Nevertheless, the existence of nematic fluctuations across such a wide temperature and doping range suggests that they are a fundamental ingredient to describe the normal state of the system (11).

Fig. 3. Temperature dependence of the dimensionless nematic susceptibility of $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ for various compositions (open symbols). Successive data sets are offset vertically by 75 for clarity. Solid lines are fits based on a phenomenological Ginzburg-Landau theory, taking into account an intrinsic built-in strain (13).

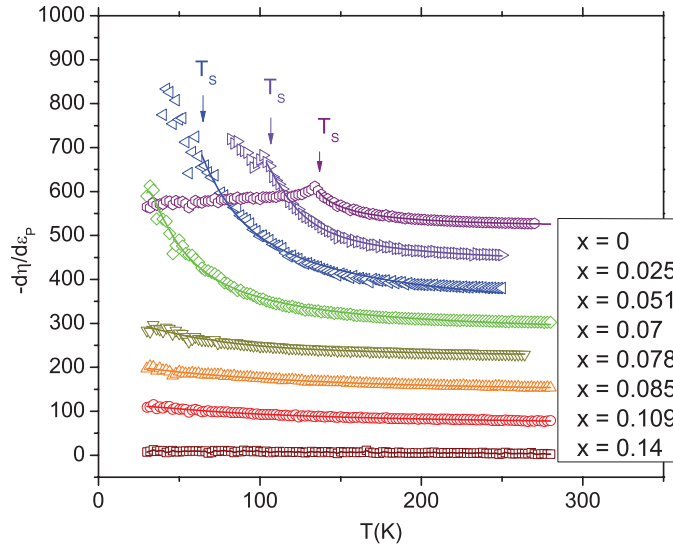
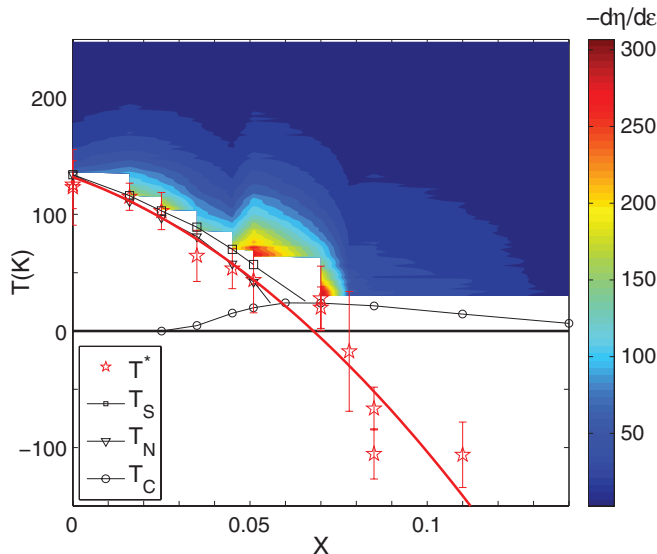


Fig. 4. Evolution of the nematic susceptibility ($d\eta/d\varepsilon$) of $\text{Ba}(\text{Fe}_{1-x}\text{Co}_x)_2\text{As}_2$ as a function of temperature and doping. Structural, magnetic, and superconducting transition temperatures (T_s , T_N , and T_c) are shown as squares, triangles, and circles. The mean field electronic nematic critical temperatures (T^*) obtained from the fit to the data in Fig. 3 are shown as open red stars. Error bars are obtained by varying the fitting temperature range (13). The evolution of nematic susceptibility and nematic critical temperatures indicates that an electronic nematic quantum phase transition occurs close to optimal doping.



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Exceptional Activity for Methane Combustion over Modular Pd@CeO₂ Subunits on Functionalized Al₂O₃

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There is a critical need for improved methane-oxidation catalysts to both reduce emissions of methane, a greenhouse gas, and improve the performance of gas turbines. However, materials that are currently available either have low activity below 400°C or are unstable at higher temperatures. Here, we describe a supramolecular approach in which single units composed of a palladium (Pd) core and a ceria (CeO₂) shell are preorganized in solution and then homogeneously deposited onto a modified hydrophobic alumina. Electron microscopy and other structural methods revealed that the Pd cores remained isolated even after heating the catalyst to 850°C. Enhanced metal-support interactions led to exceptionally high methane oxidation, with complete conversion below 400°C and outstanding thermal stability under demanding conditions.

Methane (CH₄) is the largest constituent of natural gas and is widely used in power generation and in other heating applications. However, the release of unburned CH₄ during homogeneous combustion is a serious problem, given that CH₄ is a greenhouse gas with an effect that is 20 times higher than that of CO₂. Presently available, emissions-control catalysts are notoriously ineffective at reducing concentrations of CH₄ in exhaust streams. High-temperature combustion also results in the emission of toxic nitrogen oxides (NO_x) and CO. Combustion of CH₄ promoted by heterogeneous catalysts could utilize the available energy of methane at lower temperatures, increasing system performance and limiting emissions by drastically reducing the required temperatures (1).

Given the high stability of CH₄, heterogeneous catalysts for methane oxidation must be very active at low reaction temperatures (below at least 400°C). Furthermore, materials for this application must also be catalytically and mechanically stable at high reaction and flame temperatures (2). PdO_x supported on alumina or zirconia is recognized as one of the best catalysts for catalytic CH₄ oxidation (3), even if the active phase of the catalysts is still disputed. Unfortunately, Pd-based catalysts tend to deactivate through loss of active surface by sintering and by transformation into metallic Pd at temperatures above 600°C (4). Both experimental and theoretical studies reveal that ceria (CeO₂) can improve the catalytic activity of supported Pd by stabilizing PdO_x (5–8), but pure CeO₂ has limited thermal stabil-

ity. Other systems based on metal oxides have been studied, but their activity is generally much lower, with complete CH₄ conversion obtained only above 600°C (2). Materials that could simultaneously enhance the performance of Pd-based catalysts at low temperatures and limit deactivation at elevated temperatures would greatly improve catalytic CH₄ combustion processes.

The tailored positioning of the building blocks at the nanometer scale can markedly improve the performance of the materials through electronic and steric interactions. Heterogeneous catalysts that are used in a wide variety of industrial and environmental applications already take advantage of the synergy between a support and the supported phases. Some oxides, such as ceria, can participate in the catalytic cycle by providing reactive oxygen through formation of vacancies (9, 10). In this case, it is essential that the catalytic sites be located in close proximity to the interface area between the metal particles and the oxide support. Indeed, dual-site mechanisms, where reactants are activated at the metal-support interface, are known to exist (11).

Core-shell structures are special cases in which metal-support interactions are enhanced by maximizing this interface (12). The strong interactions between the components of the core and the shell can lead to advanced materials for catalytic (13, 14) and photocatalytic (15) applications. Besides the possibility of improved catalytic performance, the self-assembly, core-shell approach offers a powerful tool for minimizing deactivation of the catalyst by metal sintering processes (12, 16). These phenomena are particularly pronounced for high-temperature reactions, as is the case of CH₄ combustion (7).

Here, we report on a hierarchical design of core-shell type catalysts inspired by the concepts of supramolecular chemistry (17) in which the building blocks are preorganized in a way to exploit their catalytic interactions to the maximum extent. Supramolecular chemistry concepts have not been widely applied in heterogeneous cat-

alysis because of the difficulty in manipulating the metal-support interaction at the nanoscale. Two active building blocks, Pd and CeO₂, were prepared separately, then self-assembled and organized in solution to form supramolecular core-shell structures held together by metal ion–ligand coordination interactions. We exploited the preorganization of the functionalized Pd@CeO₂ core-shell structures to disperse single units onto a modified, catalytically inert alumina carrier. Transmission electron microscopy (TEM) revealed that single isolated structures were deposited and maintained even after severe thermal treatments at temperatures up to 850°C. The special configuration of the hierarchical catalyst led to exceptionally high and stable performance for the catalytic combustion of methane with reduced amounts of Pd and ceria. This particular geometry appears to stabilize the active phase of the catalyst, suppressing agglomeration of the metal particles upon high-temperature calcination and increasing the concentration of PdO_x.

The supramolecular Pd@CeO₂ core-shell structures were prepared according to our recently published procedure (18). This method is based on the self-assembly between functionalized metallic Pd particles (~2 nm) protected by 11-mercaptopundecanoic acid (MUA) and a Ce(IV) alkoxide. It takes advantage of a strategic combination of interactions; the first of these occurs between the thiol group of MUA and Pd, and the second one is between the carboxyl group of the MUA and Ce(IV) moieties. A controlled hydrolysis in the presence of dodecanoic acid of the resulting assembled units leads to the formation of the Pd@CeO₂ structures, in which the CeO₂ shell is composed of small crystallites (~3 nm) organized around the preformed Pd particles. The structures are dispersible in common low-polarity solvents such as tetrahydrofuran, dichloromethane, toluene, and other hydrocarbons and are amenable for controlled deposition onto different substrates. Furthermore, the extension of this procedure to other core-shell compositions [Pd and Pt as core; TiO₂, ZrO₂, and CeO₂ as shells (19)] gives to the present approach a wide applicability and versatility.

We previously demonstrated that the Pd@CeO₂ structures can be deposited onto pristine, commercial alumina, resulting in redox properties and catalytic performances different from those of conventional or bulk materials (20). However, because pristine alumina is highly hydrophilic (21), we observed minimal interactions between the alumina support and the hydrophobic Pd@CeO₂ structures, so that the Pd@CeO₂ structures tended to agglomerate with one another rather than adhering to the support (Fig. 1A). This agglomeration was confirmed by high-angle annular dark field (HAADF)–scanning transmission electron microscopy (STEM) images collected at different tilting angles [figs. S1 and S2 (22)]. The active-phase agglomeration may introduce the generation of hot spots and deactivate the catalyst by sintering, so it was crucial to develop a synthetic

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strategy able to deposit the Pd@CeO₂ as single units on the support.

The alumina surface was first made hydrophobic by reacting it with an organosilane, triethoxy(octyl)silane (TEOOS) (Fig. 1B) (23). Because this silane has three alkoxy groups that are prone to hydrolysis and one alkyl chain that is not, the reaction between the silane and alumina can lead to one of the following two situations (24): Either the silanol groups formed by hydrolysis of the ethoxy ligands can react with OH groups of the alumina surface to form oxane bonds of the type Si-O-Al, or the silane molecules can react with each other to give multimolecular structures of bound silanes on the surface. In both cases, the strong Si-C bond ensures that the hydrophobic chain is attached to Si moieties, causing the surface of alumina to be covered by alkyl chains. In addition, the presence of Si can also benefit the reducibility of the supported CeO₂ (25). A movie of water droplets (colored by means of FeCl₃ for clarity) poured on a powdery layer of both pristine and hydrophobic alumina demonstrates the efficiency of the adopted strategy [movie S1 (22)]. The water droplets deposited on the pristine alumina immediately spread on the powder as a consequence of the favorable interactions with the alumina OH groups. By contrast, the water droplets deposited on the hydrophobic alumina were repulsed. Fourier-transform infrared analysis confirmed the formation of alkyl chain attachments onto the surface of alumina [fig. S3 (22)].

Hydrophobic Al₂O₃ (hereafter referred to as H-Al₂O₃) exhibited much greater capacity for the adsorption of the Pd@CeO₂ structures compared to the pristine, hydrophilic Al₂O₃. The adsorption resulted in a color change of the supernatant solution [fig. S4 (22)], which was almost colorless when adsorbed onto hydrophobic alumina but dark when adsorbed onto pristine alumina. To quantitatively measure the adsorption of Pd@CeO₂

structures on H-Al₂O₃, we measured the absorbance at 500 nm for a solution of Pd@CeO₂ after the addition of varying amounts of H-Al₂O₃. Because the solution of Pd@CeO₂ structures shows a broad absorption band in the ultraviolet-visible region (the Pd to CeO₂ weight ratio was fixed at 1:9) [inset of fig. S5 (22)], the concentration of Pd@CeO₂ structures remaining in solution can be inferred from the intensity of the absorption. The absorbance of the supernatant versus loading curve showed a characteristic sigmoidal shape, with a sharp increase for attempted loadings greater than 1 weight % (wt %), indicating that the H-Al₂O₃ surface became saturated at coverages higher than this. Notably, this loading is approximately half of that expected for a theoretical monolayer, assuming the Pd@CeO₂ structures pack in a close-packed configuration over the entire available surface area [likely because only one-half of the surface area of the H-Al₂O₃ is associated with mesopores that have a diameter smaller than that of the Pd@CeO₂ units, ~15 nm in dimension as prepared, preventing these pores from contributing to the adsorption process [fig. S6 (22)]]. The deposition of Pd@CeO₂ onto H-Al₂O₃ also led to the formation of pores with diameters smaller than 10 nm that were not present in the original H-Al₂O₃ [inset of fig. S6, part B (22)]. These pores could be associated with the Pd@CeO₂ units themselves (18). The porous nature of the CeO₂ shell is indeed corroborated by CO chemisorption data (see below), which demonstrates the accessibility of Pd. The requirement of having the proper support pore sizes for deposition of Pd@CeO₂ onto the alumina was further demonstrated by our attempts to deposit these structures onto hydrophobic Fe₂O₃ and SiO₂ samples, materials with narrow pore-size distributions but smaller pore size than Al₂O₃ [fig. S7 (22)]. With both hydrophobic Fe₂O₃ that had an average pore diameter of 13 nm and SiO₂ that had an average pore diameter of 4 nm, very little adsorption

of the Pd@CeO₂ structures was observed, despite the very high surface area in the SiO₂ support.

Several electron microscopy techniques were used to demonstrate that single Pd@CeO₂ supramolecular structures were successfully deposited onto the hydrophobic alumina (Fig. 2). HAADF-STEM images (Fig. 2, A, B, and D) show Pd@CeO₂ as small bright spots on the underlying surface of the hydrophobic alumina crystallites. The Pd@CeO₂ units are well dispersed and well separated throughout the entire supporting material. Images collected at different tilting angles confirmed that the structures were indeed single units [fig. S1 (22)]. X-ray energy dispersive spectroscopy (EDS) analysis with a very fine probe (0.5 nm) confirmed that the bright spots are composed of Pd and Ce with the correct, initial weight ratio (Fig. 2C). By analyzing more than 50 single spots, we found both Pd and Ce to be associated in 49 of 50 spot analyses, thus demonstrating that the core-shell structures are intact and do not segregate after the deposition and calcination to 850°C. One spot showed the presence of only CeO₂ (spot 3 of Fig. 2C); a small concentration of CeO₂ nanoparticles may have been produced in the initial synthesis, or excess ceria on the Pd@CeO₂ particles may have been removed during the calcination of the supported catalyst to 850°C. After the calcination at 500°C, EDS line profiles clearly revealed single Pd@CeO₂ structures, showing that the Pd signal arose from the core (Fig. 2E); high-resolution electron microscopy (Fig. 2F) further confirmed a core-shell structure. White boxes in Fig. 2F highlight a single Pd@CeO₂ particle, and selected digital diffraction patterns demonstrate the presence of Pd in the core and of ceria in the outer layer. CeO₂ crystallites were ~3 nm in size, in agreement with line broadening of the powder x-ray diffraction lines (fig. S8). These small Pd crystallites were maintained even after calcination at 850°C, and this stabilization was almost certainly

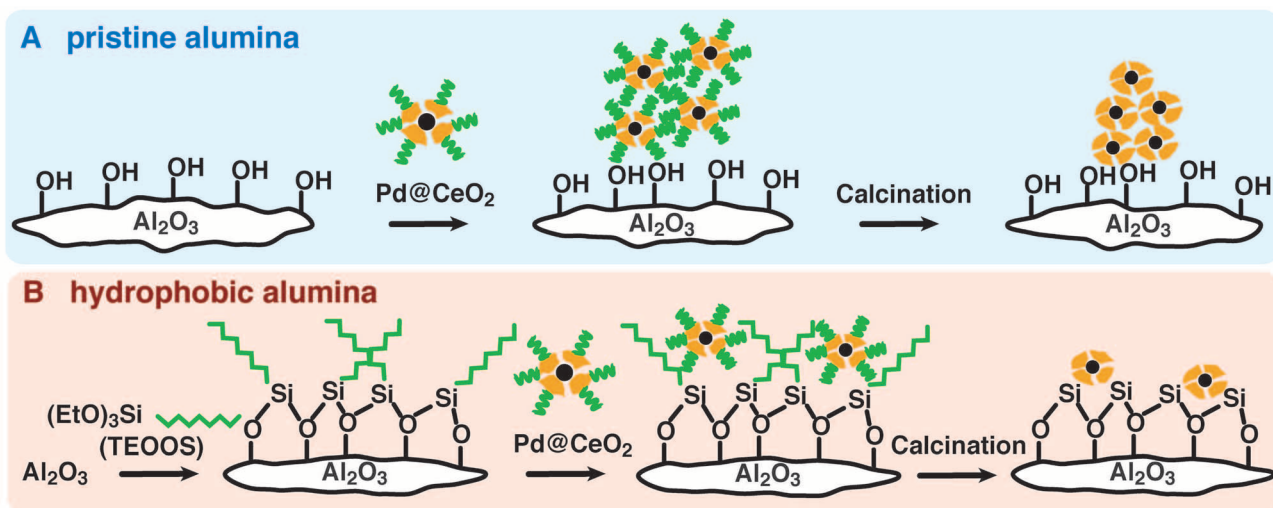


Fig. 1. Schematic representation of the agglomeration of Pd@CeO₂ structures when using the pristine alumina (A) and their deposition as single units after treatment of the same support with triethoxy(octyl)silane (TEOOS) (B).

a result of the core-shell configuration, where the organization of the crystallites around the pre-formed Pd particles avoids their agglomeration. In any case, Pd was always associated with a surrounding CeO_2 layer, so that there was no indication that the Pd@CeO_2 particles had decomposed. Furthermore, although the CeO_2 shell is porous, the results suggest that intimate contact between the components can reduce the occurrence of Ostwald ripening (see also below).

A model of the Pd@CeO_2 units that are present on our support is shown in movie S2. The structure, which is formed by a central Pd nanoparticle (about 1.8 nm in diameter) surrounded by 11 CeO_2 nanocrystals, has the expected final weight ratios (1 and 9%, respectively). In some orientations, the Pd nanoparticle is completely hidden by the surrounding ceria nanocrystals, demonstrating the difficulty of imaging these structures by microscopy. The microscopy data provide conclusive evidence that the core-shell structure of the single Pd@CeO_2 units remains intact and show that these structures possess a high thermal stability upon deposition on the hydrophobic alumina.

The $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalysts were tested for the combustion of CH_4 ($\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$). To compare the effect of the nanostructure on the catalytic activity, we prepared additional reference samples using conventional synthetic procedures. The first reference catalyst consisted of 1 wt % Pd on a CeO_2 support, prepared by optimized incipient wetness impregnation (denoted as $\text{Pd/CeO}_2\text{-IWI}$). A second reference sample was prepared by impregnation of Pd (at 1 wt

%) and CeO_2 (at 9 wt %) from their nitrate salts onto pristine alumina (denoted as $\text{Pd/CeO}_2/\text{Al}_2\text{O}_3\text{-IMP}$). These and two additional reference samples are described in the supplementary materials (fig. S9). All of the catalysts were calcined at 850°C for 5 hours and tested under the same reaction conditions.

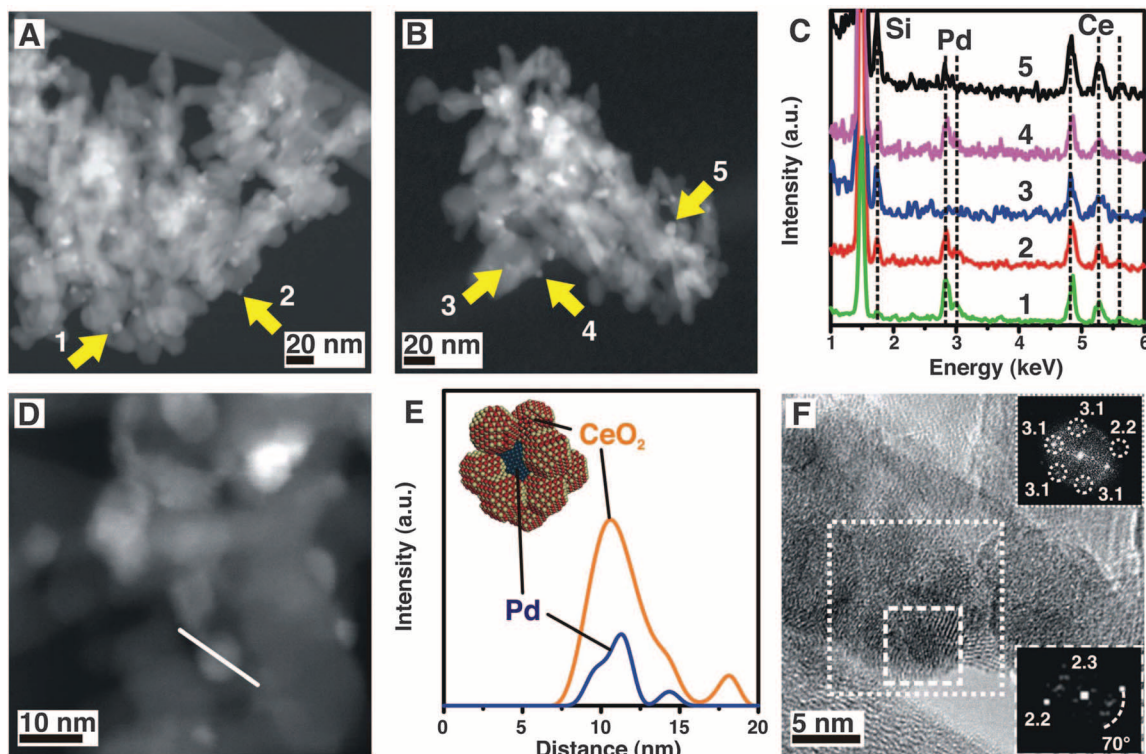
CO chemisorption experiments confirmed the accessibility of the Pd phase in all the catalysts (table S1). The thermal stability of the $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalyst against sintering was confirmed by the average Pd particle size after calcination at 850°C (2.2 nm) being very close to that of the initial starting Pd nanoparticles. The $\text{Pd/CeO}_2/\text{Al}_2\text{O}_3\text{-IMP}$ sample demonstrated poor thermal stability and had an average Pd particle size of 6.0 nm after calcination. The $\text{Pd/CeO}_2\text{-IWI}$ sample exhibited a small average particle size (1.9 nm), in accordance with previous reports for materials obtained by using similar preparation methods (7, 26). The $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalysts prepared with different loadings of the structures (Pd/Ce weight ratio was kept at 1/9) showed similar metal dispersions as measured by CO chemisorption (table S1), in accordance with the molecular nature of the Pd@CeO_2 units.

The $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ material demonstrated outstanding catalytic performance. Complete conversion of CH_4 was observed for a gas stream of 0.5 volume % CH_4 and 2.0 volume % O_2 in Ar at a space velocity of $200,000 \text{ ml g}^{-1} \text{ hour}^{-1}$ at about 400°C (Fig. 3). By comparison, all the other reference samples achieved complete CH_4 conver-

sion only above 700°C (fig. S9), more than 300° higher than that found with the $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalyst. Even when compared to state-of-the-art Pd/CeO_2 systems under the same reaction conditions, the temperature of complete conversion is decreased by more than 130°C (7). The enhanced reactivity of the $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalyst is almost certainly the result of the strong Pd- CeO_2 interaction of the core-shell Pd@CeO_2 units. These interactions are not as optimal in the $\text{Pd/CeO}_2\text{-IWI}$ catalyst, whereas some Pd may not even be in contact with CeO_2 in the $\text{Pd/CeO}_2/\text{Al}_2\text{O}_3\text{-IMP}$ sample, resulting in lower activities when compared to the $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalyst.

PdO_x is commonly recognized as the active phase for this reaction (1). In the 650° to 850°C temperature range, PdO decomposes to the thermodynamically stable Pd metal, which is much less active (1, 3). The formation of metallic Pd decreases the rates for CH_4 combustion and is commonly observed as a transient decrease in the CH_4 conversion in light-off curves for both supported and unsupported Pd-based systems (3). The nature of the support can modify this behavior, and the presence of CeO_2 can shift the temperature window in which this transition occurs, provided that there is good contact between Pd and ceria (6). $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ showed a stable activity for CH_4 oxidation over the entire range of temperatures studied (250° to 850°C) (Fig. 3A), with no decrease in activity during either heating or cooling curves. By contrast, the reference samples clearly show the usual transient decrease in CH_4 conversion, both during the

Fig. 2. TEM investigations of Pd@CeO_2 core-shell structures dispersed on hydrophobic alumina. (A) HAADF-STEM image after calcining to 500°C for 5 hours and (B) to 850°C for 5 hours. (C) EDS spot analysis of the indicated particles. (D) High-magnification HAADF-STEM image of the $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalysts calcined to 500°C and (E) the corresponding EDS line profile together with a model [see also supplementary materials and movie S2]. (F) HRTEM image of a single Pd@CeO_2 structure on the $\text{Pd@CeO}_2/\text{H-Al}_2\text{O}_3$ catalysts calcined to 500°C . The digital diffraction patterns of the particles in the white squares are reported in the top-right and bottom-right insets together with representative bond distances ($\text{\AA}$) and bond angles for Pd and ceria.



heating and the cooling portions of the curves at temperatures between 600° and 750°C, in agreement with previous reports (4). To our knowledge, such strong inhibition of the dip deactivation in the conversion curve in Pd-based catalysts for catalytic CH₄ oxidation has not been observed previously, a result that again points to a special role of the CeO₂ in the core-shell configuration in stabilizing the active phase of the catalyst. The maximized metal-support interface area and the well-known oxygen donation capability of CeO₂ can favor the oxidation of Pd nanoparticles, sustaining the catalytic reaction in the entire range of investigated temperatures.

To gain further insights, we conducted temperature-programmed oxidation experiments on the three samples (fig. S10). A PdO-Pd transition is observed in each of the samples, but is shifted to higher temperatures on the Pd@CeO₂/H-Al₂O₃ sample. Also, there is a direct relationship between the amount of oxygen released in the upward temperature ramp and taken up in the cooling ramp and the sample activity. This indicates that transformation of metallic Pd into PdO_x is maximized in the supramolecular catalyst due to the close contact of ceria with Pd, explaining the much improved activity of Pd@CeO₂/H-Al₂O₃. Indeed, there was only a very small decrease in activity for the Pd@CeO₂/H-Al₂O₃ sample during cooling, even under extremely demanding reaction conditions (gas hourly space velocity of ~1,000,000 ml g⁻¹ hour⁻¹) (fig. S11). Furthermore, the Pd@CeO₂/H-Al₂O₃ was stable to aging treatments at 850°C for 12 hours (fig. S12) and after subsequent run-up and cool-down experiments (fig. S13). CO chemisorption results on the Pd@CeO₂/H-Al₂O₃ sample, performed after catalytic tests (table S1), showed minimal evidence for Pd sintering and no evidence for re-dispersion of PdO, ruling out the contribution of this effect to the observed high, stable activity.

There are a number of possible explanations for why the ceria shell has such a pronounced effect in maintaining an oxidized Pd core. First, the thin ceria shell could well be under mechanical stress due to spatial confinement of individual Pd@CeO₂ units. It has recently been demonstrated that stress can positively affect the oxygen mobility (27). Second, the small CeO₂ crystallite size that is maintained as a result of the templating effect of the Pd cores likely leads to a high degree of disorder within the ceria shell, breaking the typical fluorite structure that stabilizes Ce⁴⁺ and thereby increasing the reducibility of the ceria shell (28). Third, the decoration of the Pd by ceria is not complete, because there is still appreciable adsorption of CO. This could lead to the formation of a high concentration of undercoordinated, reactive Pd sites at the interface between the metal and the oxide that are known to be more effective in CH₄ activation (29).

Kinetic rate data further corroborate the very high intrinsic activity of the supramolecular catalyst when compared to the reference catalysts (Fig. 4). The reaction rates on the Pd@CeO₂/H-

Al₂O₃ sample were about 40 times higher than those on Pd/CeO₂-IWI and 200 times higher than on Pd/CeO₂/Al₂O₃-IMP, respectively, under the same experimental conditions (Fig. 4A). Furthermore, the rates were more than one order of magnitude higher than that of other optimized Pd-based catalysts (7). CO adsorption data (table S1) demonstrated that the difference in activity cannot be related to the amount of exposed Pd because the Pd/CeO₂-IWI sample showed a higher Pd accessibility than that of the Pd@CeO₂/H-Al₂O₃ core-shell catalyst (60% versus 50%, respectively). The apparent activation energies for each of the catalysts were also similar (90 to 120 kJ mol⁻¹) and slightly lower than those in the literature (7), but implying that the nature of the active sites in Pd@CeO₂/H-Al₂O₃ are similar to that of the other

two catalysts. Notably, the number of active sites was markedly increased in the Pd@CeO₂/H-Al₂O₃ sample by means of the special configuration, as evidenced by the larger preexponential factor and turn-over-frequency values (table S2). Furthermore, samples prepared at different Pd loadings (Pd/Ce weight ratio was maintained at 1/9) showed very similar reaction rates when normalized by the amount of metal (Fig. 4B) and exhibited identical activation energies (100 to 110 kJ mol⁻¹).

The data presented here demonstrate that the Pd@CeO₂ structures deposited as single units on the hydrophobic alumina act as supramolecular catalysts. In these structures, the synergy between Pd and CeO₂ produces active sites that are equally active in all of the samples, though in different

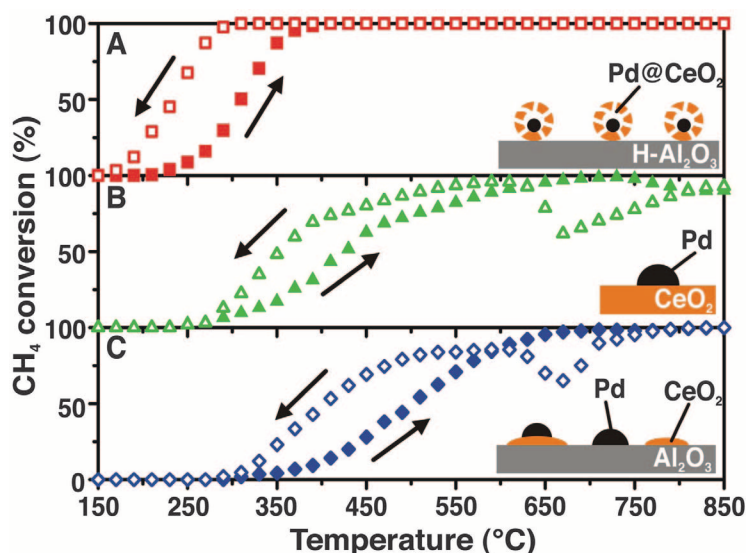


Fig. 3. Heating and cooling (10°C min⁻¹) light-off curves of CH₄ conversion against the temperature for the three catalyst formulations used. (A) Pd@CeO₂/H-Al₂O₃ core-shell catalyst, (B) Pd/CeO₂-IWI, and (C) Pd/CeO₂/Al₂O₃-IMP.

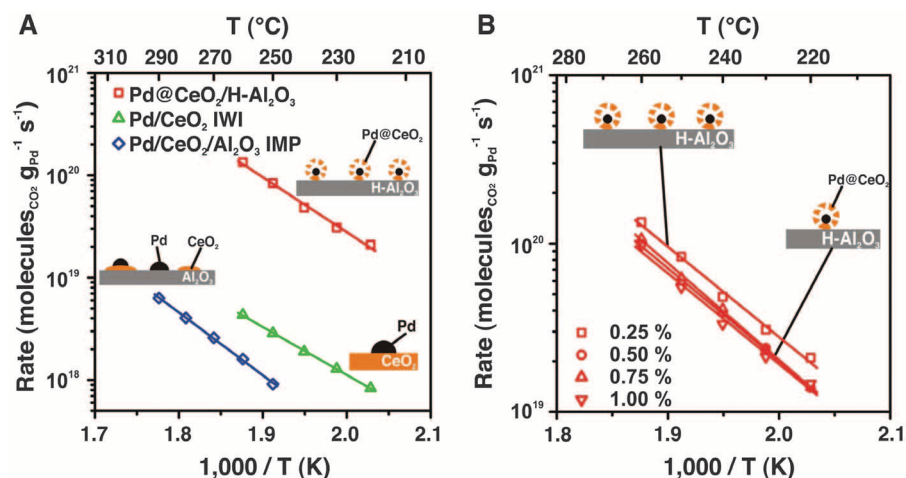


Fig. 4. Kinetic rate data for CH₄ oxidation on (A) Pd@CeO₂/H-Al₂O₃ core-shell catalyst, Pd/CeO₂-IWI, and Pd/CeO₂/Al₂O₃-IMP; (B) Pd@CeO₂/H-Al₂O₃ core-shell catalysts at different loadings of the structures (Pd/Ce weight ratio was kept at 1/9): Pd loading of 0.25, 0.50, 0.75, and 1.00%.

numbers. As a further confirmation, CO chemisorption results demonstrated very similar Pd accessibility for all of the Pd@CeO₂ samples prepared (table S2), corroborating the defined geometry and morphology obtained through the supramolecular approach. This approach could potentially be valuable even for three-way catalysts, where the special properties shown here could be important for improving the activity at low oxygen concentrations, enhancing stability against sintering, and protecting against poisoning through the core-shell configuration.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S13

Tables S1 and S2

Movies S1 and S2

References (30–33)

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Synthesis and Structure of a Terminal Uranium Nitride Complex

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The terminal uranium nitride linkage is a fundamental target in the study of f-orbital participation in metal-ligand multiple bonding but has previously eluded characterization in an isolable molecule. Here, we report the preparation of the terminal uranium(V) nitride complex [UN(Tren^{TIPS})] [Na(12-crown-4)]₂ {in which Tren^{TIPS} = [N(CH₂CH₂NSiPr₃)₃]³⁻ and Prⁱ = CH(CH₃)₂} by reaction of the uranium(III) complex [U(Tren^{TIPS})] with sodium azide followed by abstraction and encapsulation of the sodium cation by the polydentate crown ether 12-crown-4. Single-crystal x-ray diffraction reveals a uranium-terminal nitride bond length of 1.825(15) angstroms (where 15 is the standard uncertainty). The structural assignment is supported by means of ¹⁵N-isotopic labeling, electronic absorption spectroscopy, magnetometry, electronic structure calculations, elemental analyses, and liberation of ammonia after treatment with water.

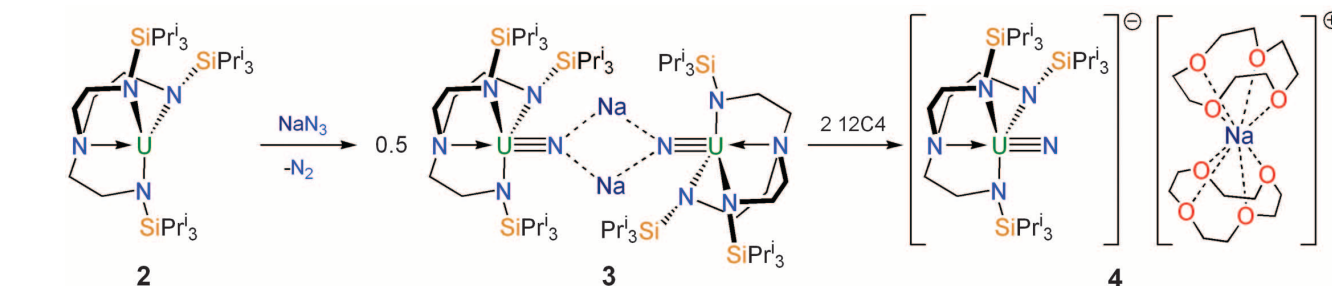
Multiple bonding to uranium is exemplified by well-established U=O and U=NR double bonds in uranyl and im-

ido complexes (1), respectively, and exploration of U=CR₂ double bonds in carbene derivatives now represents a burgeoning field (2–4). None-

theless, prominent by their paucity are molecular U≡N triple bonds in uranium nitride complexes. A thorough understanding of the physicochemical properties of the uranium nitride linkage is key to predicting the long-term stability and reactivity of [UN]_n as a ceramic nuclear fuel and to the ongoing debate regarding the nature and extent of uranium 5f and 6d valence orbital participation in uranium-ligand multiple bonding (5). Furthermore, understanding the inherent reactivity of the uranium nitride linkage with respect to electrophiles and nucleophiles is a prerequisite to identifying viable catalytic cycles (6). However, little is known about the intrinsic reactivity of the uranium nitride linkage because of the difficulties associated with synthesizing and solubilizing this functional group. Uranium nitrides

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Scheme 1. Synthesis of compounds **3** and **4** from precursor **2**.

are typically prepared through the reduction of dinitrogen or ammonia with uranium under reducing conditions, often at high temperatures and pressures (7, 8). Unfortunately, the harsh reaction conditions introduce impurities that are difficult to remove, and thus in recent years, attention has focused on using low-temperature and hence more controllable molecular routes to access uranium nitrides.

The only reports of terminal uranium nitrides pertain to UN, NUN, and UNF₃ prepared under matrix isolation conditions at 4 K (9–11) and the [NUO]⁺ cation observed with mass spectrometry (12, 13). Molecular uranium nitride complexes that have been isolated under ambient conditions in exploitable quantities exclusively exhibit polymetallic assemblies, in which the nitride center bridges two (14–16), three (17, 18), or four (19) metal ions or is protected by a covalently linked borane group that is impervious to removal (20). The transient formation of a uranium nitride during photolysis of uranium(IV) azides has been reported, but under photochemical conditions, indiscriminate insertion of a U–N group into C–H bonds with loss of dinitrogen was observed (21).

We report here the successful synthesis and characterization of a terminal uranium(V) nitride complex. Our strategy combined a very sterically demanding polydentate triamidoamine ligand with a two-electron oxidation using sodium azide, followed by abstraction and encapsulation of the weakly bound sodium ion.

We first designed and prepared the very sterically demanding uranium(IV) precursor complex [UCl(Tren^{TIPS})] [1, Tren^{TIPS} = [N(CH₂CH₂NSiPr₃)₃]³⁻; Prⁱ = CH(CH₃)₂], which we reduced over a potassium mirror in order to afford the trivalent uranium complex [U(Tren^{TIPS})] (2) as dark purple crystals in 78% yield (22). Uranium(III) is strongly reducing, and the crystal structure of 2 (22) reveals a well-defined pocket at uranium where a cylindrical molecule could ligate to the coordinatively unsaturated metal center (fig. S8). We therefore reasoned that the reaction of 2 with sodium azide would effect a two-electron redox reaction to produce a sodium-coordinated nitride functionality, with concomitant elimination of dinitrogen. Because the chemical bonding of sodium is highly ionic in nature, we anticipated that although sodium coordination to the nitride group would provide electrostatic stabilization during preparation—thus precluding deleterious side reactions—subsequent sequestration of the loosely bound sodium ion would straightforwardly afford a terminal uranium nitride functionality under mild conditions.

Treatment of 2 with one equivalent of sodium azide in pyridine resulted in the elimination of dinitrogen, and after work-up, red crystals of the dimeric nitride complex [{U(μ-N)(μ-Na)(Tren^{TIPS})₂}]₂ (3) were isolated from toluene in 60% yield (Scheme 1) (22). The Fourier transform infrared (FTIR) spectrum of 3 exhibits a U≡N stretch at 955 cm⁻¹. This band is reduced in intensity by

~50% for ¹⁵N-3 prepared from 2 and 98% 1-¹⁵N-labeled sodium azide, accompanied by the appearance of a band at 930 cm⁻¹. This compares well

with the calculated isotopomer shift to 925 cm⁻¹ from reduced mass considerations. The molecular structure of 3 as determined with x-ray crystal-

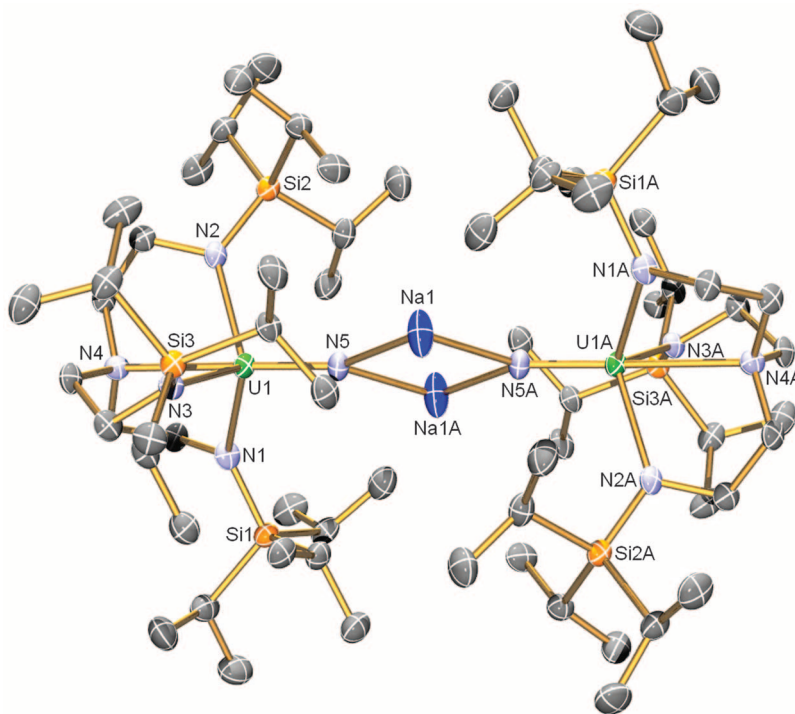


Fig. 1. Molecular structure of crystalline [{U(μ-N)(μ-Na)(Tren^{TIPS})₂}]₂ (3) at 90 K with 50% probability ellipsoids. Hydrogen atoms are omitted for clarity. Selected distances and angles are U1–N1, 2.333(4) Å; U1–N2, 2.322(4) Å; U1–N3, 2.328(4) Å; U1–N4, 2.654(4) Å; U1–N5, 1.883(4) Å; N5–Na1, 2.308(5) Å; Na1–N5A, 2.345(5) Å; N4–U1–N5, 174.12(16)°; N1–U1–N2, 109.33(15)°; N1–U1–N3, 111.38(15)°; N2–U1–N3, 106.56(15)°; N5–Na1–N5A, 99.26(17)°; and Na1–N5–Na1A, 80.74(17)°.

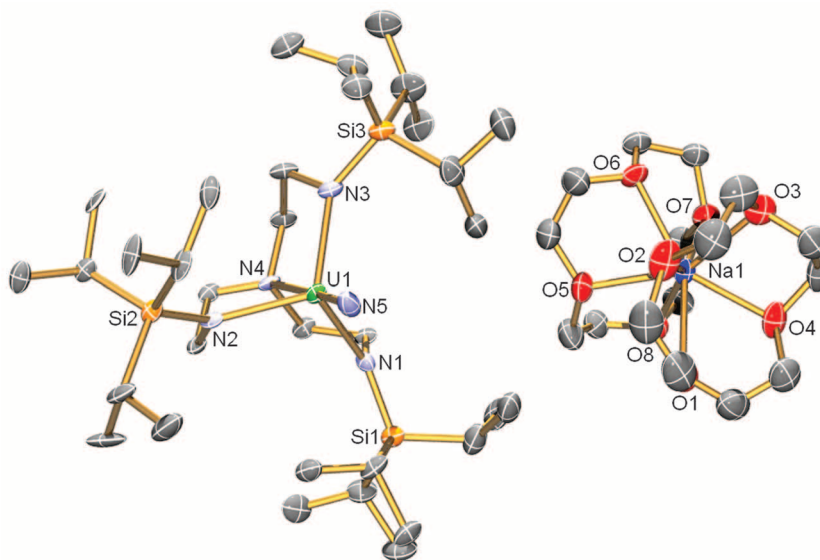


Fig. 2. Molecular structure of crystalline [UN(Tren^{TIPS})] [Na(12C4)₂] (4) at 90 K with 50% probability ellipsoids. Hydrogen atoms are omitted for clarity. Selected distances and angles are U1–N1, 2.328(15) Å; U1–N2, 2.365(15) Å; U1–N3, 2.367(14) Å; U1–N4, 2.713(13) Å; U1–N5, 1.825(15) Å; Na1–O1, 2.468(19) Å; Na1–O2, 2.41(2) Å; Na1–O3, 2.445(19) Å; Na1–O4, 2.379(18) Å; Na1–O5, 2.432(16) Å; Na1–O6, 2.431(16) Å; Na1–O7, 2.504(16) Å; Na1–O8, 2.491(16) Å; N4–U1–N5, 178.0(7)°; N1–U1–N2, 108.0(5)°; N1–U1–N3, 107.2(5)°; N2–U1–N3, 110.1(5)°.

lographic studies is shown in Fig. 1. The salient feature of centrosymmetric **3** is the presence of two uranium nitride groups that are mutually bridged by two sodium cations. The apparently two-coordinate sodium cations supplement their coordination spheres with several agostic-type interactions to neighboring isopropyl C-H bonds ($\text{Na}\cdots\text{HC} = 2.407$ to 2.588 Å). The remaining coordination sphere of the two uranium centers is dominated by the very sterically demanding tetradentate $\text{Tren}^{\text{TIPS}}$ ligands, which enforce distorted trigonal bipyramidal geometries at each uranium center. The uranium-nitride distances were determined to be $1.883(4)$ Å (where the value in parentheses represents the standard uncertainty), which compare with a $\text{U}\equiv\text{N}$ bond length of $1.916(4)$ Å in the uranium(V) borane-capped nitrido complex $[(\text{C}_6\text{F}_5)_3\text{BNU}(\text{NArBu})_3][\text{N}(\text{Bu}^n)_4]$ [$\text{Ar} = 3,5\text{-(CH}_3)_2\text{C}_6\text{H}_3$, $\text{Bu}^t = \text{C(CH}_3)_3$, and $\text{Bu}^n = (\text{CH}_2)_3\text{CH}_3$] (20) and terminal uranium(V) imido bond lengths that typically average 1.95 Å (23). The uranium-amide and -amine bond lengths in **3** are surprisingly similar to values observed in **1** (22). We suggest that this is a result of the removal of essentially nonbonding 5f electrons during oxidation of **2** (16) combined with the very sterically demanding and rigid nature of the $\text{Tren}^{\text{TIPS}}$ ligand framework preventing close approach of the nitrogen centers to uranium. The nitride-sodium distances of $2.308(5)$ and $2.345(5)$ Å are shorter than the mean $\text{Na-N}_{\text{amide}}$

distance of 2.437 Å from the literature (23). This may reflect the undoubtedly electron-deficient nature of the sodium atoms in **3** and also suggests a nucleophilic nitride center.

To test the hypothesis that the bridging sodium cations in **3** would be labile because of the ionic nature of the sodium-nitride interactions, we treated **3** with two molar equivalents of the cyclic crown ether 12-crown-4 (12C4) per sodium cation and obtained red blocks of the complex $[\text{UN}(\text{Tren}^{\text{TIPS}})][\text{Na}(\text{12C4})_2]$ (**4**) in 43% yield after work-up (Scheme 1) (22). The FTIR spectrum of **4** is less clear-cut than for **3** owing to overlapping bands from the 12C4 ether in the fingerprint region. However, an absorption at 936 cm^{-1} is assigned as a $\text{U}\equiv\text{N}$ stretch because for $^{15}\text{N-4}$, prepared from $^{15}\text{N-3}$ and 12C4 ether, this band is reduced in intensity by $\sim 50\%$, and the band structure at $\sim 900\text{ cm}^{-1}$ grows in intensity. Furthermore, this shift is in agreement with a calculated isotopomer shift to 906 cm^{-1} from reduced mass considerations. Confirmation of abstraction and encapsulation of the sodium cation from **3** to afford a separated ion pair motif in **4** was afforded by the x-ray crystal structure (Fig. 2). The crystal data for **4** reveal a terminal uranium-nitride distance of $1.825(15)$ Å. This represents a modest but significant contraction of $0.06(2)$ Å compared with **3** and may reflect the weak nature of the sodium-nitride interactions in **3**. With respect to $\text{Tren}^{\text{TIPS}}$ coordination, the uranium-amide distances

in **4** are remarkably similar to **3**, but the uranium-amine distance of $2.713(13)$ Å in **4** is longer than the corresponding distance in **3** [$2.654(4)$ Å]. This reflects the fact that the uranium center in **4** resides 0.824 Å above the plane defined by the three amide centers, whereas in **3**, the corresponding displacement is 0.791 Å for each uranium ion.

The ultraviolet/visible/near-infrared (UV/Vis/NIR) electronic absorption spectra of **3** and **4** (figs. S6 and S7) are dominated by charge transfer bands that tail in from the high-energy portion of the spectrum to $\sim 13,000\text{ cm}^{-1}$. For $5f^1$ uranium(V), spin-orbit coupling splits the 2F orbital manifold into two J multiplets (where J is the total angular momentum): $J = 5/2$ and $7/2$ ground and excited terms, respectively. Each of these J levels is split further by the ligand crystal field into $(2J+1)/2$ doubly degenerate levels so that in C_{3v} symmetry, the $5/2$ multiplet splits into two $\mu = 1/2$ ($^2\Gamma_4$) and one $\mu = 3/2$ ($^1\Gamma_5+^1\Gamma_6$) Kramers doublets, and the $7/2$ multiplet splits into three $\mu = 1/2$ ($^2\Gamma_4$) and one $\mu = 3/2$ ($^1\Gamma_5+^1\Gamma_6$) doublets, where μ is the crystal quantum number. On the assumption that the spin orbit coupling constant of 2174 cm^{-1} (24) for uranium(V) is considerably larger than the crystal field splitting, four optical transitions would be expected in the NIR region. For **3** and **4**, in addition to weak vibronic bands, broad and featureless Laporte forbidden $f \rightarrow f$ transitions ($\epsilon = 8$ to 20 cm^{-1}) are indeed observed. The transitions for **4** are blue-shifted compared with **3**, but overall, the energies of these transitions compare favorably with data reported for terminal uranium(V) oxo complexes (25).

The pentavalent assignments of **3** and **4** were confirmed by means of variable-temperature superconducting quantum interference device (SQUID) magnetometry (Fig. 3). A powdered sample of **4** exhibits a magnetic moment of $1.99\text{ }\mu_B$ (where μ_B is in Bohr magneton units) at 298 K ($2.20\text{ }\mu_B$ in solution). This is lower than the theoretical magnetic moment of $2.54\text{ }\mu_B$ for one uranium(V) center but is in agreement with reported uranium(V) magnetic moments more generally (26). Characteristic of uranium(V), the magnetic moment of **4** decreases slowly, until at 50 K ($\mu_{\text{eff}} = 1.82\text{ }\mu_B$), the moment drops more rapidly, reaching $1.31\text{ }\mu_B$ at 1.8 K . For comparison, the magnetic moment of **3** at 298 K was found to be of $3.20\text{ }\mu_B$ per dimer unit, decreasing slowly with decreasing temperature until 100 K ($2.45\text{ }\mu_B$) and then falling quickly to a value of $1.16\text{ }\mu_B$ at 1.8 K . The room-temperature moment per uranium ion in **3** ($2.26\text{ }\mu_B$) is only slightly lower than the theoretical value of $2.54\text{ }\mu_B$ for the free uranium(V) ion. These data are quite distinct from those of tetravalent **1** and trivalent **2**, which exhibit magnetometric profiles that are consistent with their respective oxidation states; most tellingly, the magnetic moment of **1** at 298 K is $2.01\text{ }\mu_B$, but this decreases to $0.50\text{ }\mu_B$ at 1.8 K , whereas for **2** the magnetic moment at 298 K of $3.12\text{ }\mu_B$

Fig. 3. Temperature-dependent SQUID magnetization data for tetravalent **1** (black triangles), trivalent **2** (blue circles), pentavalent dimeric nitride **3** (green squares), and pentavalent terminal nitride **4** (red diamonds) measured over the temperature range of 1.8 to 300 K .

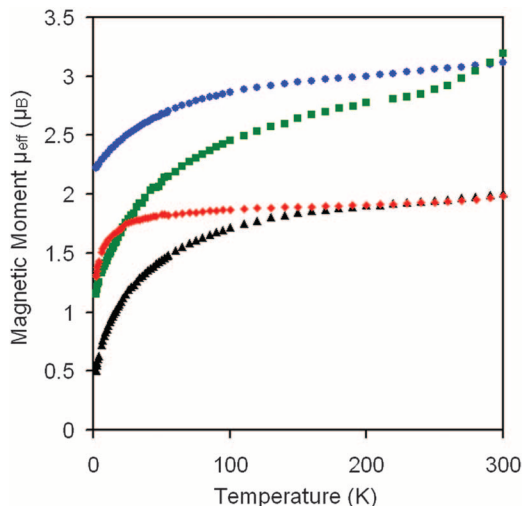
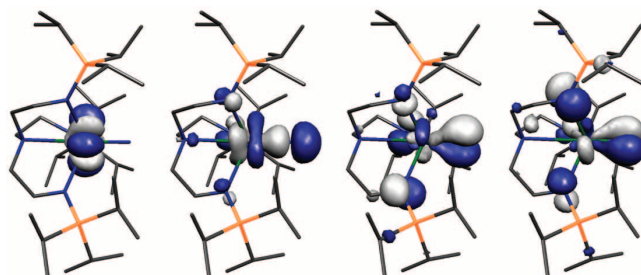


Fig. 4. The top four occupied α -spin Kohn Sham molecular orbital representations calculated for **4** at the $0.05\text{ e } \text{\AA}^3$ level with hydrogen atoms omitted for clarity. From left to right they are HOMO ($222a$, 0.952 eV), HOMO-1 ($221a$, -1.261 eV), HOMO-2 ($220a$, -1.547 eV), and HOMO-3 ($219a$, -1.552 eV).



decreases to $2.22 \mu_B$ at 1.8 K. The low-temperature (5 K) electron paramagnetic resonance (EPR) spectrum of powdered **2** measured at 4 GHz (22) is typical of uranium(III) ($g_{\text{eff}} = 3.9, 1.8, 0.6$). However, extensive solid-state and frozen-solution EPR measurements of **3** and **4** show no significant EPR spectral features down to 5 K. This EPR-silent behavior implies that the lowest Kramers doublet in the $^2F_{5/2}$ ground term for **3** and **4** is the EPR-inactive $\mu = 3/2$, as is the case for certain uranium(V) imido complexes (25, 26).

In order to probe the nature of the uranium-nitride linkages in **3** and **4**, we calculated the electronic structures of the full dimer of **3** and the anion $[\text{UN}(\text{Tren}^{\text{TIPS}})]^-$ fragment of **4** using density functional theory (DFT). The DFT calculations agree very well with experiment, with bond lengths and angles predicted to within 0.02 Å and 2° of experiment, respectively. The molecular orbitals (MOs) involved in the terminal $\text{U}\equiv\text{N}$ triple bond in **4** are illustrated (Fig. 4), and the calculations clearly show the involvement and importance of the 5f orbitals. The most energetic electron in **4** is of essentially pure, nonbonding 5f character and resides in the highest occupied molecular orbital (HOMO), which is consistent with the uranium(V) formulation of **4**. The next three MOs (HOMOs $-1, -2, -3$) exhibit substantial interactions between the uranium and nitride centers in **4** and together comprise the $\text{U}\equiv\text{N}$ triple bond. In essence, the same bonding picture is calculated for **3** (fig. S10), except the dimeric nature of **3** results in delocalized pairs of MOs representing the nonbonding 5f orbitals and σ - and π -MOs from the $\text{U}\equiv\text{N}$ triple bonds in **3**. For both **3** and **4**, the σ -component is higher in energy than the two quasi-degenerate π -components. This π -, π -, σ -MO ordering is the same as for the uranyl dication (27) but is reversed compared with the usual energetic ordering of σ - then π -orbitals that are typically observed in $\text{U}=\text{C}$ and $\text{U}=\text{N}$ double bonds and chemical bonding manifolds more generally (28). This may reflect an antibonding interaction at short U-N distances between (for a $\text{U}\equiv\text{N}$ bond orientated along the z axis) the σ -orientated nitrogen $2p_z$ orbital and the annular lobes of the uranium 6d and 5f orbitals (27, 29).

The valence MOs of **3** and **4** are delocalized over the complexes. In order to obtain a localized and more chemically relevant description of the $\text{U}\equiv\text{N}$ triple bonds in **3** and **4**, natural bond orbital (NBO) analyses were performed (figs. S12 and S13). For **4**, NBO analysis (table S4) reveals a σ -bond containing 32% uranium and 68% nitrogen character. In turn, the uranium component is composed of 5% 7s, 4% 7p, 44% 5f, and 47% 6d orbital character, and the nitrogen component is composed of 7% 2s and 93% 2p character. For the two π -components, each bond is composed of 27% uranium and ~73% nitrogen character, and the latter contribution contains exclusively nitrogen 2p orbital character, reflecting the π -nature of the bonding of these MOs. The uranium component to the π -bonds com-

prises 72% 5f and 28% 6d character, with no meaningful 7s or 7p contributions. A similar bonding manifold is apparent for **3**, except the uranium contributions to the σ - and π -components of the $\text{U}\equiv\text{N}$ triple bond are typically 7% lower than in **4**, reflecting polarization of nitride electron density from the $\text{U}\equiv\text{N}$ triple bond toward the two bridging sodium ions in **3**. The calculated charge at uranium in **4** is +3.34, and the spin density is -1.26 , which suggests donation of electron density to uranium from the ligands (table S3). For comparison, a purely ionic bonding picture would yield a formal charge of +5 and a spin density of -1 at uranium. The calculated charge on the nitride nitrogen center is -1.36 , which suggests a nucleophilic nitride as experimentally manifested by the coordination to sodium cations in **3**. The uranium nitride Mayer bond order of 2.91 for **4** is consistent with a formal $\text{U}\equiv\text{N}$ triple bond, compared with $\text{U}-\text{N}_{\text{amine}}$ and average $\text{U}-\text{N}_{\text{amide}}$ Mayer bond orders of 0.29 and 0.65, respectively, for $\text{Tren}^{\text{TIPS}}$ coordination in this complex. In contrast, the uranium nitride Mayer bond order of 2.21 in **3** is lower than in **4**, which reflects depletion of the $\text{U}\equiv\text{N}$ bond order as a result of the aforementioned polarizing interactions with the two electropositive sodium cations. Taken together, these observations are consistent with the emerging bonding picture for U-N and U-O bonds, which describes the former as exhibiting more covalent bonding as compared with the latter (28).

Ether solutions of **4** decompose rapidly to give intractable mixtures, which is consistent with the previous paucity of terminal uranium nitride linkages. Preliminary reactivity studies of **4** show it to be reactive. Complex **4** reacts with excess water (15 equivalents so as to ensure complete consumption of **4**) in the presence of three equivalents of cobaltocene to afford ammonia in $41 \pm 5\%$ yield ($10 \pm 5\%$ in the absence of cobaltocene), confirming the presence of a basic nitride linkage in **4** (22, 30). Furthermore, complexes **3** and **4** have considerable potential as starting materials for the synthesis of a wide range of uranium-nitrogen multiple-bond linkages. For example, reaction between **4** and Me_3SiCl in toluene results in formation of $[\text{Na}(\text{12C}_4)_2][\text{Cl}]$ and the corresponding uranium(V) imido complex $[\text{U}(\text{NSiMe}_3)(\text{Tren}^{\text{TIPS}})]$ (**5**), the latter of which has been independently prepared and verified by reaction of **2** with Me_3SiN_3 (22). Considering that a diuranium- μ_2 -nitride has been shown to exhibit electrophilic reactivity (16), but formation of **5** suggests a nitride with nucleophilic character in **4**, detailed studies of the chemistry of **3** and **4** will enable a comprehensive assessment of the nature of uranium-nitrogen multiple bonds in order to rationalize and understand their inherent structure-bonding-reactivity relationships.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1223488/DC1
Materials and Methods
Figs S1 to S13
Tables S1 to S7
References (31–41)

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The Provenances of Asteroids, and Their Contributions to the Volatile Inventories of the Terrestrial Planets

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Determining the source(s) of hydrogen, carbon, and nitrogen accreted by Earth is important for understanding the origins of water and life and for constraining dynamical processes that operated during planet formation. Chondritic meteorites are asteroidal fragments that retain records of the first few million years of solar system history. The deuterium/hydrogen (D/H) values of water in carbonaceous chondrites are distinct from those in comets and Saturn's moon Enceladus, implying that they formed in a different region of the solar system, contrary to predictions of recent dynamical models. The D/H values of water in carbonaceous chondrites also argue against an influx of water ice from the outer solar system, which has been invoked to explain the nonsolar oxygen isotopic composition of the inner solar system. The bulk hydrogen and nitrogen isotopic compositions of CI chondrites suggest that they were the principal source of Earth's volatiles.

Two recent dynamical models of the orbital evolution of the outer planets (1, 2), designed to explain the small mass of Mars and the late heavy bombardment, predict that the objects in the asteroid belt have two sources: S-complex asteroids formed in situ, whereas D-type, P-type, and C-complex asteroids formed either between the giant planets or in the trans-Neptunian region. Chondrites, the most primitive meteorites, are fragments of main-belt asteroids and are divided into several classes (ordinary, OC; Rumuruti, RC; enstatite, EC; and carbonaceous, CC) that are subdivided into numerous groups. On the basis of their reflectance spectra and other evidence, the OCs and CCs have been linked to the S- and C-complex asteroids, respectively. It was suggested (2) that D- and P-type asteroids are the sources of primitive micrometeorites, rather than meteorites. However, most micrometeorites appear to be related to CI and CM CCs (3), and at least one ungrouped CC, Tagish Lake, has been spectroscopically linked to D-type asteroids (4). At present, there is no evidence that a major type of primitive asteroid is unrepresented in our meteorite collections (3). There is isotopic evidence for a difference in the provenances of the OC-EC-RCs and the CCs (5), but there are no reliable indicators of where in the solar nebula they formed.

The proposed formation locations for the D-type, P-type, and C-complex asteroids are potentially the same as those of comets—Oort cloud comets are thought to have formed in the giant planet region and the trans-Neptunian region, while Jupiter-family comets (JFCs) probably formed in the trans-Neptunian region (6). Although chondrites share many similarities with comets (3), they do not now contain ice. The CCs, as well as the OCs and RCs, accreted water, presumably as ice, but the record of this is now preserved only in hydrated silicates (7, 8). Perhaps the CC parent bodies were larger than typical comets, so that internal heating caused much of the water to be consumed by alteration of silicates, Fe-metal, and sulfides and what remained sublimed away in the ~4 to 4.5 billion years that they have resided in the inner solar system. Nevertheless, if CCs have a cometary heritage, a record of it should be preserved in the hydrogen isotopes of their hydrated silicates.

In a static solar nebula, radial temperature gradients, combined with equilibrium and kinetic factors, mean that water D/H values should have increased with increasing formation distance from the Sun (9, 10). Given its dynamic evolution, the spatial and temporal variations in ice compositions in the solar nebula are likely to have been more complex. Nevertheless, objects that formed in the same source regions and at similar times should have accreted ice with similar hydrogen isotopic compositions. Therefore, a comparison of water D/H values in comets and other icy outer solar system bodies with CCs is potentially a direct test of the predictions of the dynamical models.

Hydrogen isotopic compositions are available for the water in some comets and Saturn's icy moon Enceladus. The D/H values of the OH in hydrated silicates in chondrites are likely to have been modified during alteration from the water compositions at the time of accretion. The

chondrites also accreted organic matter (11), and the hydrogen in it further complicates the determination of the original isotopic compositions of their water (3). Here, we report the bulk hydrogen, carbon, and nitrogen elemental and isotopic compositions of 86 samples of chondrites (table S1) and adopt two approaches to use them for estimating or placing limits on the original hydrogen isotopic compositions of their water (3).

In the first approach, we analyzed CCs from the CM and CR groups that experienced different degrees of aqueous alteration. If the bulk hydrogen isotopic compositions are simply the products of mixing between hydrated silicates and organic matter, then, in a plot of δD versus C/H, the bulk compositions should form a line with the hydrogen isotope intercept giving the isotopic composition of the water. In Fig. 1, the data for the CMs and CRs do fall on lines, and the implied δD values for their water are $-444 \pm 23\text{‰}$ and 96^{+110}_{-65}‰ , respectively [$\delta D = 1000 \times (R_{\text{sample}}/R_{\text{standard}} - 1)$, where R_{standard} is the D/H value of standard mean ocean water]. The subparallel CM and CR trends converge on the region where the most primitive insoluble organic material (IOM) plots (Fig. 1B). This implies that the CMs and CRs shared a common primitive organic component. The IOM in most chondrites is less deuterium-rich than that in the most primitive meteorites (Fig. 1B), suggesting that there has been hydrogen isotope exchange between the water and the organics in essentially closed systems.

The CIs and the most primitive COs, CVs, and OCs are not common enough to use the same approach. The two CIs (Orgueil and Ivuna) and the CO (ALH 77307) analyzed here fall on the CM trend, suggesting that they all probably had similar initial water compositions. Three lithologies from Tagish Lake exhibit different degrees of alteration (12). Their bulk compositions show limited variations in δD and C/H and suggest that the water in Tagish Lake had a slightly lower D/H value than in the CMs. The bulk hydrogen isotopic composition of the OC Semarkona (Fig. 1B) has a much higher D/H value than any of the CCs.

Because all chondrites, not just the CMs and CRs, probably accreted a common organic component (11), the second approach for estimating the water compositions is by subtraction of the organic component. The hydrogen isotopic compositions of the water in CMs and CRs can be estimated, albeit with larger errors (3), by assuming that (i) the bulk carbon is in material with IOM-like C/H ratios and (ii) it has an isotopic composition like that of the most primitive IOM ($\delta D \approx 3500\text{‰}$). This approach has been used (3) to estimate the ranges of likely water compositions in the CIs, COs, CVs, Tagish Lake, and the OCs (Fig. 2 and table S2). The ranges reflect whether the measured IOM C/H values are used, or a more primitive ratio based on the CR IOM that may better represent the IOM

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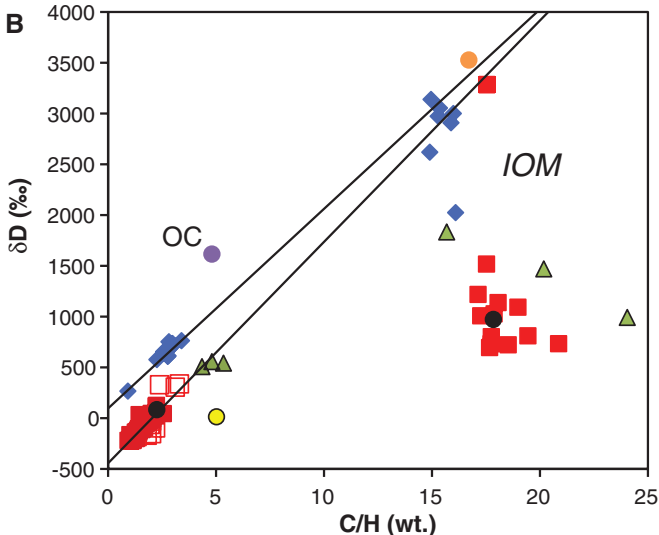
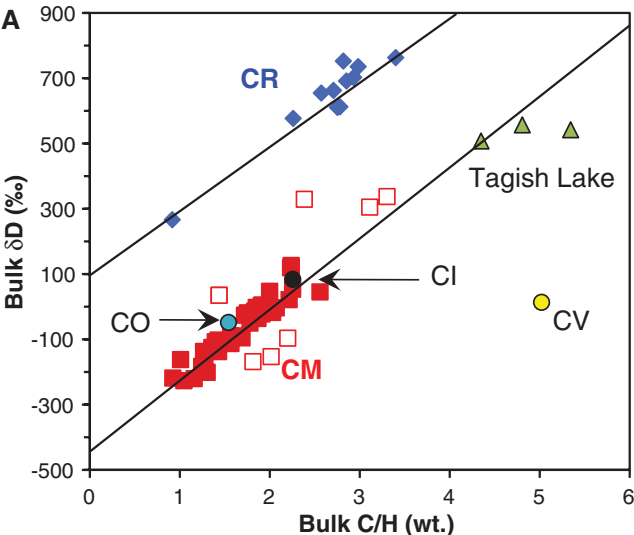


Fig. 1. (A) The bulk compositions of the carbonaceous chondrites analyzed here. The lines are fits to the CR and CM data. Open red squares are CMs that were not included in the CM fit. **(B)** An expanded scale to show

that the CR and CM lines pass through the compositions of the most primitive chondritic insoluble organic matter (IOM) (11, 14). The bulk composition of the OC Semarkona is also included.

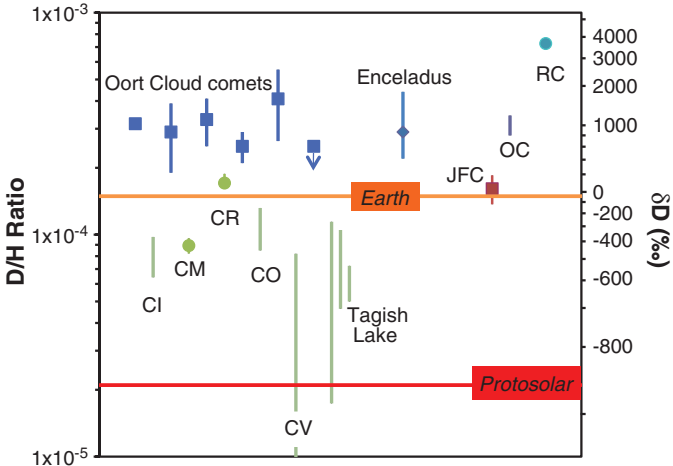
composition at the onset of alteration and metamorphism (11).

The six measured Oort cloud comets and Enceladus have water D/H values that are enriched in deuterium by roughly a factor of 2 compared to Earth and more than an order of magnitude relative to the initial solar composition (Fig. 2). The one measured JFC has a water hydrogen isotopic composition that is similar to Earth's (13). This suggests that there may not be a monotonic rise in the D/H values of icy bodies with formation distance from the Sun. Nevertheless, the D/H value of this JFC is still enriched in deuterium by almost an order of magnitude compared to the initial solar composition. Consequently, one would still expect that water accreted by asteroids that formed in the asteroid belt (e.g., S-complex asteroids) would have lower D/H values than objects that formed in the giant planet region and beyond (e.g., C-complex asteroids).

The OC water hydrogen isotopic composition is similar to that of Oort cloud comets [the RCs are more deuterium enriched (8)], whereas the CR water composition is like that of Hartley-2 (Fig. 2). The water compositions of the CIs, CMs, CO, CV, and Tagish Lake are less deuterium-rich than any measured comets, implying formation closer to the Sun. The lower deuterium enrichments in CCs than in OCs and RCs also run counter to the typically assumed order of formation distances for the chondrites (3). However, the hydrogen isotopic compositions in Fig. 2 and table S2 should be regarded as upper limits because isotopic fractionation associated with the observed oxidation of Fe by water during alteration and metamorphism is likely to have enriched the residual water in deuterium (14).

There has been speculation that some CCs are cometary in origin. On the basis of estimates of their preatmospheric orbits, it has been sug-

Fig. 2. Comparison of the estimated hydrogen isotopic compositions of water in various chondrite groups with those measured in Oort cloud and Jupiter family comets (JFC), and Saturn's icy moon Enceladus. See (3) for details and sources.



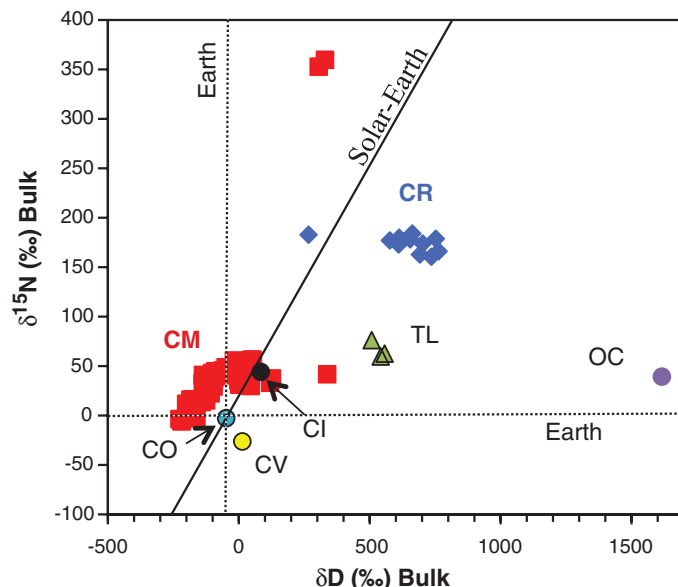
gested that Orgueil (CI) is a fragment of a JFC (15) and that the CM Maribo is related to comet P2/Encke (16). So-called main-belt comets with typical asteroidal orbits have also recently been discovered (17). Hence, the suggestion that many outer main-belt asteroids (and therefore CCs) formed in the same regions as conventional comets is attractive, but the subterrestrial D/H values of the water in most CCs do not support this. However, most Oort cloud comets may have formed in the trans-Neptunian region, not the interplanetary region, in which case, the Oort comet-like D/H value of Enceladus provides the only constraint for the possible interplanetary origin (1) for the CCs. Enceladus appears to have accreted from material that formed in the solar disk (18), rather than in the dense subnebula around Saturn when it was growing rapidly. This suggests that the sources of the CC parent bodies were sunward of Saturn's orbit (3 to 7 astro-

nomical units) as it approached its final mass (1), including in the asteroid belt.

All analyzed bodies in the terrestrial planet region (within the orbit of Jupiter), including the Earth and chondrites, have oxygen that is enriched in ¹⁷O and ¹⁸O, compared to the bulk solar system, represented by the solar wind (19). The favored explanation is an early, massive influx of ¹⁶O-poor water ice that formed either in the outer solar system or the presolar molecular cloud (20, 21). The bulk and internal oxygen isotopic systematics of OCs and CCs are at least consistent with accretion of ¹⁶O-poor ices (22, 23). However, our results are in conflict with the ice influx explanation because outer solar system ice should have D/H values like those of cometary ices and molecular cloud ices are even more deuterium-rich (24, 25).

The range of D/H values in chondritic water could be explained in the context of the ice influx

Fig. 3. The bulk hydrogen and nitrogen isotopic compositions of chondrites (TL, Tagish Lake). The line connects the solar and terrestrial isotopic compositions. Bodies similar to chondrites are potential sources of Earth's volatiles. For reasons discussed in the text, CI-like material with ~10% contributions of material with isotopically solar compositions, but a roughly chondritic H/N value, can most simply explain Earth's bulk hydrogen and nitrogen isotopic compositions (3).



model if variable amounts of the accreted water had reequilibrated at high temperatures with nebular H_2 , thereby acquiring roughly solar hydrogen isotopic compositions. One obvious local heat source is chondrule formation, in which case one might expect an inverse correlation between chondrule abundances in chondrites ($OC \approx RC > CV \approx CO \approx CR > CM > \text{Tagish Lake} > CI$) and their initial water D/H values given in table S2 ($RC > OC > CR > CV \approx CO \approx CM \approx \text{Tagish Lake} \approx CI$), but this is not the case. Alternatively, the isotopically more solar-like water reequilibrated with nebular H_2 sunward of the snow line and subsequently migrated out to the chondrite-formation regions beyond the snow line—for instance, via the cold-finger effect (26). In this scenario, the water in the OCs and RCs would then be essentially pure outer solar system ice. Such an explanation is hard to understand if, as is generally assumed, the OCs and RCs formed closer to the Sun than the CCs and may have formed earlier (3). Also, there is no evidence from the internal oxygen isotope systematics of the chondrites that the water in the OCs was much more ^{16}O -depleted than in the CCs (3), as this explanation would predict. Thus, at present there is an inconsistency between the ice influx model and our results, unless, perhaps, the major ice influx occurred long before chondrite formation and the ice that chondrites accreted formed more locally (with smaller isotopic anomalies) because transport in the disk had become less efficient.

The possibility that comets may have supplied much of Earth's volatiles has been revived by the discovery that a JFC has water with an Earth-like hydrogen isotopic composition (13). However, Earth would not have accreted only cometary ice, but entire comets, including much organic material. From our knowledge of chondrites (11, 14) and interplanetary dust particles

(27) that may come from comets, this organic material is deuterium-rich. Hence, the bulk hydrogen isotopic composition of a comet is likely to be more deuterium-rich than its water (3), in which case even comets like Hartley-2 may not be viable sources of Earth's volatiles.

If comets were not the sources of Earth's water, asteroids, including main-belt comets, become the most likely candidates (28), along with some nebular gas (29). Marty (29) has argued that the relative abundances of hydrogen, carbon, and the noble gases in Earth are roughly chondritic. Nitrogen is depleted relative to carbon and hydrogen, perhaps because it is trapped in the core (i.e., the volatiles were not accreted in a late veneer) (29). If Earth's volatiles are in roughly chondritic abundances, isotopes might indicate which, if any, of the known chondrites were their major sources. The range of bulk carbon isotopic compositions of chondrites is small (3), but the hydrogen and nitrogen isotopes vary greatly (Fig. 3). No single chondrite group is identical in composition to that of the bulk Earth. The primitive CO ALH 77307 has bulk hydrogen and nitrogen isotopic compositions like Earth's, but other isotopic evidence probably rules out COs as the major source of Earth's volatiles (5). The CIs plot on the extension of the line connecting solar and bulk Earth. It is possible that a number of chondrite groups contributed to Earth's volatile budget, but perhaps the simplest explanation is that most of the hydrogen and nitrogen (as well as other volatiles) was accreted in CI-like material, along with ~10% contributions to both elements from material with a solar isotopic, but elementally fractionated, composition (3, 29).

Note added in proof: Some of the discussion regarding comparison between the meteorites and the Oort cloud comets and Enceladus has been clarified from that in the version published in *Science Express*.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1223474/DC1
Materials and Methods
Supplementary Text
Author Contributions
Figs. S1 to S3
Tables S1 to S5
References (30–113)

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Earthquake in a Maze: Compressional Rupture Branching During the 2012 M_w 8.6 Sumatra Earthquake

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Seismological observations of the 2012 moment magnitude 8.6 Sumatra earthquake reveal unprecedented complexity of dynamic rupture. The surprisingly large magnitude results from the combination of deep extent, high stress drop, and rupture of multiple faults. Back-projection source imaging indicates that the rupture occurred on distinct planes in an orthogonal conjugate fault system, with relatively slow rupture speed. The east-southeast–west-northwest ruptures add a new dimension to the seismotectonics of the Wharton Basin, which was previously thought to be controlled by north-south strike-slip faulting. The rupture turned twice into the compressive quadrant, against the preferred branching direction predicted by dynamic Coulomb stress calculations. Orthogonal faulting and compressional branching indicate that rupture was controlled by a pressure-insensitive strength of the deep oceanic lithosphere.

The 11 April 2012 moment magnitude (M_w) 8.6 earthquake off shore of Sumatra is a record-breaking event in many respects. It is the largest strike-slip and intraplate earthquake ever recorded and, as shown here, one of the most complicated ruptures ever imaged by modern seismology. The faulting geometry and the peculiarities of its complex rupture path offer a rare opportunity to probe the mechanics of the oceanic lithosphere.

The earthquake occurred in the diffuse deformation zone between the Indian and Australian plates (Fig. 1, left, inset). Its focal mechanism is typical for the region (1), with T axis normal to the Sumatra subduction trench as observed for intraplate oceanic strike-slip earthquakes elsewhere (2) and consistent with regional stress modeling (3). The rupture initiated in the Paleogene oceanic lithosphere formed at the Wharton Basin spreading center but extended unimpeded into the adjacent oceanic lithosphere affected by later volcanism on the Ninetyeast Ridge (NER).

Because of the remote offshore location of this earthquake, geodetic constraints on fault geometry and static slip for teleseismic finite source inversions are unavailable. We imaged the rupture process by means of back-projection of teleseismic data from European and Japanese seismic networks. We applied the Multitaper-MUSIC array processing technique, which provides higher resolution than that of conventional beamforming (4). We also adopted a “reference window” strategy so as to avoid the systematic “swimming” artifact (5). High-frequency (HF, 0.5 to 1 Hz) source radiation is reliably imaged during 160 s (movies S1 and S2). The methods and their resolution and uncertainty analysis are described in the supplementary materials. The

spatiotemporal evolution of the main HF sources (Figs. 1 and 2) is remarkably complex. The rupture involved at least three different, almost orthogonal, faults. Their strikes are consistent with the conjugate planes of centroid moment tensor (CMT) solutions and with the distribution of aftershocks (Fig. 1). The rupture process comprises at least three distinct stages (Fig. 1, right, inset), and the rupture length and speed on each fault are shown in Fig. 2. It started as a bilateral rupture on a fault striking WNW-ESE (“fault A”) with a rupture length of ~100 km and duration of ~25 s. This stage generated the strongest HF radiation (fig. S3). The rupture then branched into an almost orthogonal fault (“fault B”), breaking bilaterally for ~60 s over 300 km. The onset of rupture to the NNE on fault B was delayed by ~15 s and then propagated until near the Sumatra trench. Fault B’s SSW rupture front branched into a third almost orthogonal fault (“fault C”), which ruptured to the NNW for ~100 km. The final rupture stage involved stepping northward from fault C onto a parallel fault (“fault D”) that crossed the NER. The total rupture length on faults A, B, and C is 500 km, which is half that obtained by the extrapolation of empirical scaling relations (6). Two hours later, the largest (M_w 8.2) aftershock initiated on the SSE continuation of fault C but ruptured bilaterally for ~100 km on an orthogonal fault (Fig. 1, right).

The magnitude of this earthquake is surprising in an intraplate environment characterized by relatively short faults with wide stepovers. With hindsight, the large magnitude of the 2012 Sumatra earthquake stems from a conjunction of circumstances: wide depth extent, high stress drop, and rupture of multiple faults. Reported centroid depths are below 25 km [U.S. Geological Survey (USGS) CMT/W-phase solution; Global Centroid-Moment-Tensor (GCMT)]. Rupture penetrating into the uppermost mantle is consistent with old and hence thick oceanic lithosphere (~55 million years old, ~35 km) (7). West of the NER, seismic reflection lines show faults cutting through the Moho dis-

continuity (8). Considering uniform slip in a 500-km-long and 40-km-deep rupture, the estimated average slip is ~15 m, and the stress drop is ~15 MPa, which is high but similar to the stress drop of other large oceanic strike-slip earthquakes (9, 10) and not unusual for intraplate and subcrustal earthquakes (11, 12). The multisegment rupture was encouraged by stressing from the M_w 9.1 2004 Sumatra megathrust earthquake, whose southernmost large-slip region coincides with the latitude of the 2012 event (Fig. 1, left). Coulomb stress calculations show that thrust-faulting favors slip on outer-rise strike-slip faults that are oblique to the trench (13).

The dominant E-W rupture of faults A, C, and D adds a new dimension to the prevailing view of the seismotectonics of this region. These faults are subparallel to long-lived but still active faults on the NER (Fig. 3) (14). The bisecting direction of the conjugate faults is consistent with the orientation of the principal stress inferred from seismic and GPS data (15). Strike-slip focal mechanisms from the zone east of the NER have previously been attributed to slip on N-S–striking faults, such as those imaged in seismic lines south of the equator in the Wharton Basin (16). Active E-W–striking faults west of the NER are generally attributed to compressional deformation (8). The rupture geometry of this earthquake indicates that active E-W right-lateral faults are also an important part of the kinematics of this broad deformation zone.

Back-projection imaging reveals rupture on almost orthogonal faults, as confirmed with back-projection of the M_w 8.2 aftershock. This has been observed in earthquake pairs (such as 1987 M_w 6.2 Superstition Hills and M_w 6.7 Elmore Ranch; and 1992 M_w 7.3 Landers and M_w 6.5 Big Bear) but only rarely during single events, such as in the 13 May 1997 M_w 6 Kagoshima earthquake (17) and in the 2000 M_w 7.8 Wharton Basin earthquake (9), although orthogonal faulting of the latter is not confirmed by later studies (18). A multiple CMT inversion (methods are available in the supplementary materials) yields two subevents with similar mechanisms; the second one was ~200 km SW of the hypocenter (Fig. 1, left), which is consistent with rupture on the SSW branch of fault B and on fault C. In the crust, conjugate shear faults intersect at an angle of ~60°. The seafloor magnetic patterns (Fig. 1, right) rule out reactivation of fossil systems of transform faults and ridges. The wide angle between these faults requires pressure-insensitive strength during their formation (Fig. 2, inset).

The rupture path of this earthquake is unexpected: In two occasions, the rupture branched preferably into the compressive (strengthened) quadrant, with arrest or delay in the alternative branch. The NNW-ward rupture front on the right-lateral fault A first turned left into the SSW segment of fault B. Rupture on the NNE segment of fault B was delayed by ~15 s. This behavior is mirrored by the second branching episode. The SSW-ward rupture front on the

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left-lateral fault B turned right into the NNW segment of fault C. In both cases, the preferred branching direction is toward the compressive quadrant of the previous segment, which is opposite to the expectation based on usual values of friction coefficient. Analysis of the dynamic stresses induced near the tip of a right-lateral crack on orthogonal left-lateral faults (Fig. 2, right) shows that the observed branching direction requires two circumstances: low rupture speed ($V_r/V_s \sim 0.5$, where V_r is the rupture speed and V_s is the shear-wave speed) and low apparent friction coefficient (~ 0.2)—a small slope of the failure envelope in a shear-versus-normal stress diagram (Fig. 2, inset). The former is robustly supported by our back-projection results: The overall rupture speed is ~ 2.5 km/s on faults A and B (Fig. 2, left), which is not unusual compared with global average values but is slow compared with wave speeds below the oceanic Moho (50 to 60% of S wave speed) (19). The latter implies a pressure-insensitive strength, which is characteristic of ductile materials at depth. An alternative explanation by poroelastic effects (20)

with large Skempton's coefficient requires high fluid pressure that is inconsistent with the large stress drop.

Sustained seismic rupture also requires a dynamic weakening mechanism. The relatively slow rupture speed suggests scale-dependent energy dissipation by the rupture process. The ductile shear heating instability proposed by (21, 22) operates between 600 and 800°C, which is limited to a roughly 40 to 60 km depth. Serpentinized peridotite has low pressure sensitivity at confining pressures over few 100 MPa, with apparent friction coefficients as low as 0.15 (23), and might dynamically weaken by dehydration embrittlement (24). However, the serpentinization reaction is possible only up to 400 to 500°C, which corresponds to ~ 25 km depth (8). A single dynamic weakening mechanism that can operate over the whole depth range of slip of this earthquake remains to be identified.

This is not the first time an earthquake has grown larger than expected or has occurred where it is least expected. The destructive 2011 M_w 9.0 Tohoku-Oki and M_w 6.3 Christchurch earthquakes

illustrate the scientific challenge of estimating the likelihood of extreme events based on a short and incomplete historical record. The 2012 Sumatra earthquake raises the concern of similarly large events in continental strike-slip fault systems, which pose a higher hazard to populations. Although the tectonic setting in an oceanic intraplate zone of high deformation is rare, at least one of the ingredients that made this earthquake big—its large stress drop—is a general feature of other intraplate earthquakes (11). Its rupture complexity highlights the importance of considering earthquake scenarios with multisegment ruptures. The rupture transition from faults C and D across an offset larger than 20 km is particularly extreme (25). The relation to the 2004 Sumatra earthquake suggests that large outer-rise events induced by megathrust events—although not producing damaging shaking because of their remote off-shore location—can pose a tsunami hazard if they have a dip component (26) or displace high topography (27). The Gorda plate in the southern Cascadia subduction zone is such an example.

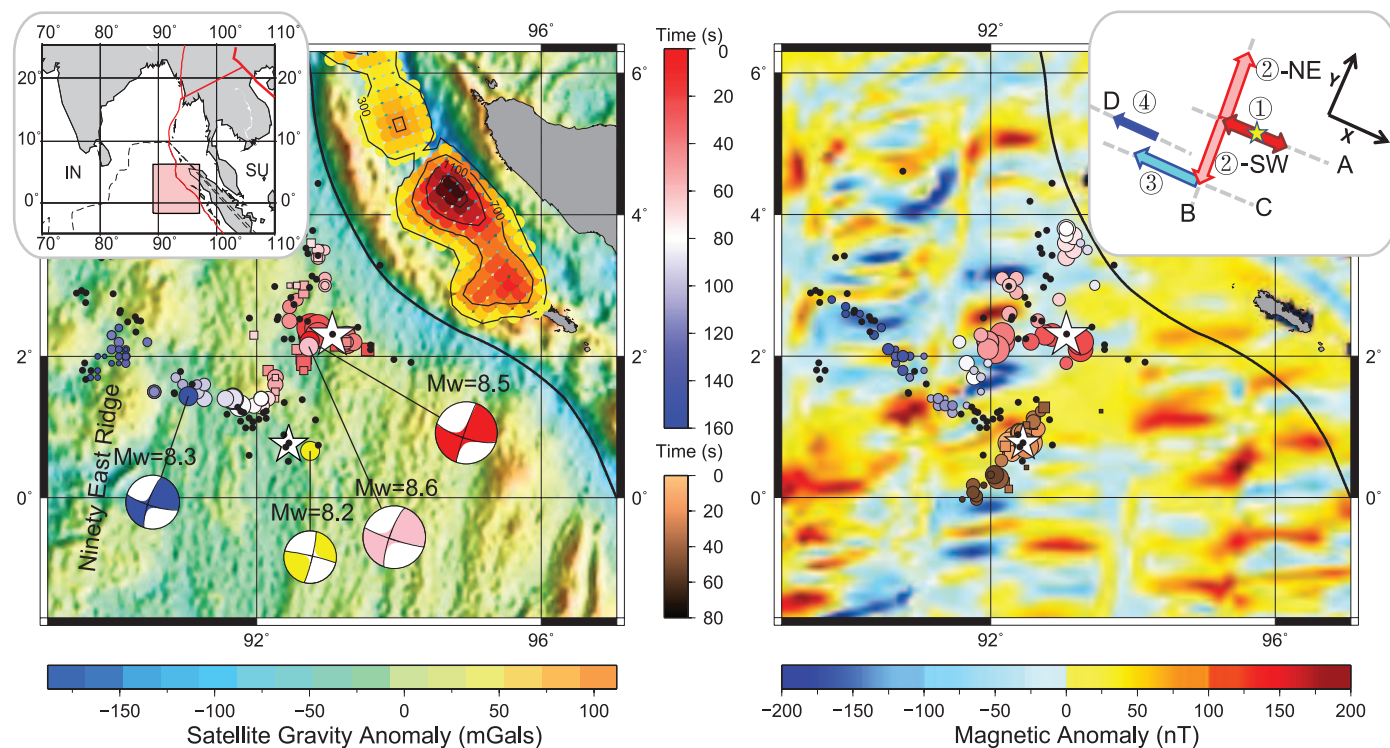


Fig. 1. Spatiotemporal distribution of HF radiation imaged by the (left) European and (right) Japanese networks. Colored circles and squares indicate the positions of primary and secondary peak HF radiation (from movies S1 and S2, respectively). Their size is scaled by beamforming amplitude, and their color indicates timing relative to hypocentral time (color scale in center). The secondary peaks of the MUSIC pseudo-spectrum are those at least 50% as large as the main peak in the same frame. The brown shaded circles in the right figure are the HF radiation peaks from the M_w 8.2 aftershock observed from Japan. The colored contours in the Sumatra subduction zone (left) represent the slip model of the 2004 M_w 9.1 Sumatra earthquake (28). The figure background is colored by the satellite gravity anomaly (left) in milligals (color

scale on bottom left) and the magnetic anomaly (right) in nanoteslas (color scale on bottom right). Black dots are the epicenters of the first day of aftershocks from the U.S. National Earthquake Information Center catalog. The big and small white stars indicate the hypocenter of the mainshock and M_w 8.2 aftershock. The moment tensors of the M_w 8.6 mainshock, M_w 8.2 aftershock, and double CMT solutions of the mainshock are shown as colored pink, yellow, red, and blue beach balls. The red line in the top left inset shows the boundary between the India (IN) and Sundaland (SU) plates (29). The patterned pink area is the diffuse deformation zone between the India and Australia plate. The red rectangular zone indicates the study area. The top right inset shows the interpreted fault planes (gray dashed lines) and rupture directions (colored arrows).

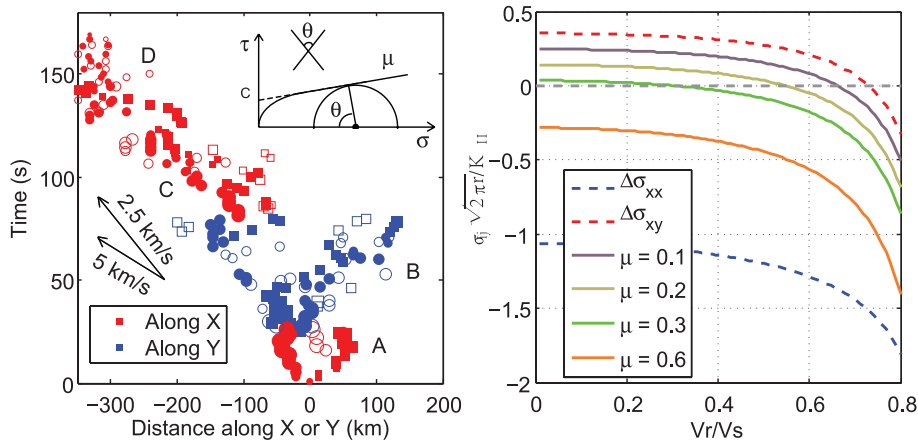
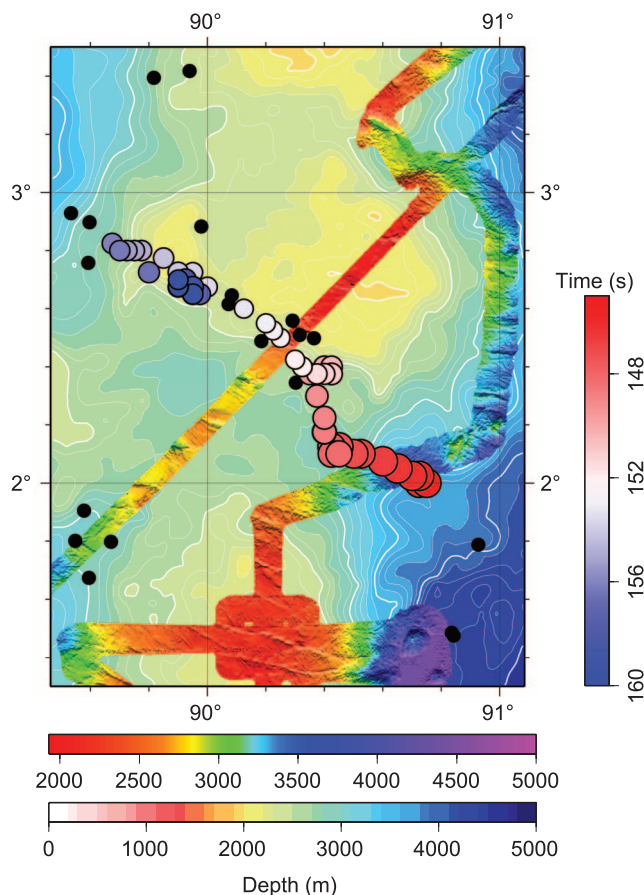


Fig. 2. Spatiotemporal details of the rupture process. **(Left)** Timing and position of the HF radiators relative to the hypocenter. The position is reported in alternation along the axes labeled X (red) and Y (blue) in Fig. 1, inset. Circles and squares are the results of Europe and Japan arrays, respectively. Solid and open symbols indicate principal and secondary HF radiators, respectively. **(Inset)** Shear strength (τ) versus normal stress (σ) diagram of a nonlinear strength envelope with small apparent friction coefficient μ (almost pressure-insensitive material) and large cohesion C , resulting in almost orthogonal failure planes ($\theta \sim 90^\circ$). **(Right)** Dynamic Coulomb stress changes induced near the tip of a right-lateral crack propagating at steady rupture speed, resolved onto orthogonal left-lateral faults in the compressional quadrant as a function of the ratio between rupture speed and shear-wave speed (V_r/V_s) (30). The symbols denote dynamic changes of normal stress ($\Delta\sigma_{xx}$, negative compressive, blue dashed line), shear stress ($\Delta\sigma_{xy}$, positive left-lateral, red dashed line), and Coulomb stress ($\Delta\sigma_{xy} + \mu\Delta\sigma_{xx}$, color solid curves, assuming various apparent friction coefficients μ indicated in the legend). Stresses are normalized based on the Mode II stress intensity factor (K_{II}) and the distance to the crack tip (r). Rupture on the compressive side can be triggered (positive Coulomb stress change) only for low enough apparent friction and rupture speed.

Fig. 3. Bathymetry where the rupture crosses the NER. Colored background is global bathymetry from SRTM30+ overlain by multibeam bathymetry from cruise KNOX06RR and cruise DYNAMO, respectively. Black dots indicate aftershocks, and circles indicate HF source radiators. These indicate rupture through the NER during the last 15 s of the earthquake. The rupture plane is consistent with numerous fault scarps visible in the multibeam bathymetry.



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Supplementary Materials

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PI4P and PI(4,5)P₂ Are Essential But Independent Lipid Determinants of Membrane Identity

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The quantitatively minor phospholipid phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P₂] fulfills many cellular functions in the plasma membrane (PM), whereas its synthetic precursor, phosphatidylinositol 4-phosphate (PI4P), has no assigned PM roles apart from PI(4,5)P₂ synthesis. We used a combination of pharmacological and chemical genetic approaches to probe the function of PM PI4P, most of which was not required for the synthesis or functions of PI(4,5)P₂. However, depletion of both lipids was required to prevent PM targeting of proteins that interact with acidic lipids or activation of the transient receptor potential vanilloid 1 cation channel. Therefore, PI4P contributes to the pool of polyanionic lipids that define plasma membrane identity and to some functions previously attributed specifically to PI(4,5)P₂, which may be fulfilled by a more general polyanionic lipid requirement.

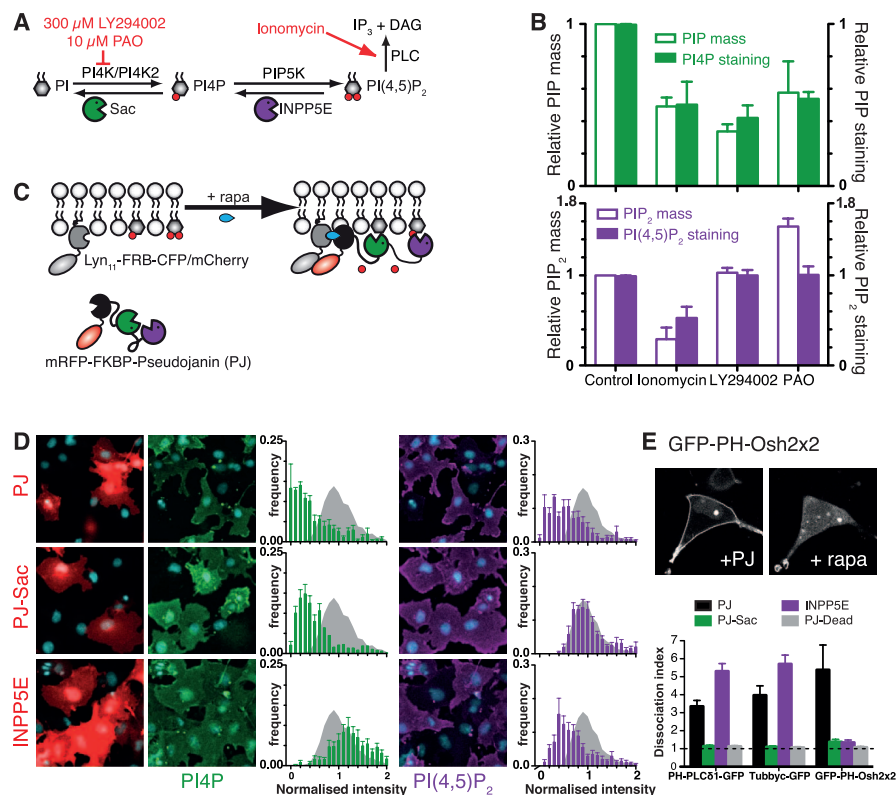
The quantitatively minor phospholipid, phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂], is found on the inner surface of the plasma

membrane (PM), where it acts as a molecular gatekeeper of both cell signaling and molecular traffic (1–3). Its major route of synthesis (Fig. 1A) is by phosphorylation of phosphatidylinositol (PI) by PI 4-kinases (PI4K or PI4K2), making phosphatidylinositol 4-phosphate (PI4P), which is then phosphorylated at the 5-position by PI4P 5-kinase (PIP5K). PI4P is generated in many cellular membranes, particularly in the Golgi apparatus, where it is crucial for function (4). Direct evidence for the presence of PI4P in the PM was scarce (5, 6), and the tacit assumption has been that it resides there solely for PI(4,5)P₂ synthesis.

Inhibitors of PI4K activity such as LY294002 and phenylarsine oxide (PAO) cause depletion of cellular PI4P, with only minor effects on the total amount of PI(4,5)P₂ (5, 7, 8). We confirmed this in COS-7 (African Green monkey fibroblast) cells using either specific immunocytochemical probes (5) or mass spectrometry (9) (Fig. 1B). As a positive control, activation of phospholipase C (PLC) with ionomycin (10) caused depletion of both lipids. Although mass spectrometry cannot distinguish regio-isomers, PI4P and PI(4,5)P₂ are the predominant isomers in mammalian cells (11).

To more selectively and acutely manipulate the abundance of PM inositol lipids, we turned to the rapamycin-inducible dimerization of FKBP (FK506 binding protein 12) and FRB (fragment of mTOR that binds rapamycin) domains, which can be used to recruit enzymes to the PM (12, 13) (Fig. 1C). We generated an enzymatic chimera of inositol polyphosphate-5-phosphatase E (INPP5E), which converts PI(4,5)P₂ to PI4P (12), and the *S. cerevisiae* sac1 phosphatase, which dephosphorylates PI4P (14). We named this fusion protein Pseudojanin (PJ), in reference to its similarity to Synaptojanin (15). PJ recruited to the PM for 2 min with rapamycin caused decreased PI4P and PI(4,5)P₂ staining (Fig. 1D) and the release of the PI4P and PI(4,5)P₂-binding Osh2 tandem pleckstrin homology (PH) domain (PH-Osh2x2) (7, 16) from the PM (Fig. 1E). Conversely, recruitment of only an INPP5E domain had no effect on PH-Osh2x2 (fig. S2), caused small increases in PI4P staining, depleted PI(4,5)P₂ staining (Fig. 1D and fig. S1E), and released PM-bound

Fig. 1. Independent depletion of PM PI4P and PI(4,5)P₂. **(A)** Synthesis of PI(4,5)P₂, and effects of inhibitors and activators. DAG, diacylglycerol. **(B)** Effect of LY294002, PAO, and ionomycin on PI4P and PI(4,5)P₂ measured by mass spectrometry (open bars) or staining (filled bars; means $\pm$ SEM, $n = 3$ to 4). **(C)** Generation of PJ, a fusion of sac and INPP5E phosphatase domains with FKBP, and its rapamycin-induced recruitment to a PM-targeted FRB domain (Lyn₁₁-FRB). CFP, cyan fluorescent protein; mCherry, a red fluorescent protein. **(D)** Effect of PJ, PJ-Sac (with inactivated INPP5E domain), or INPP5E (lacking the sac domain) on PI4P and PI(4,5)P₂ staining intensity after PM recruitment for 2 min with 1 μ M rapamycin. Histograms are means $\pm$ SEM ($n = 4$ to 5); gray peaks are the frequency of occurrence of cells with the indicated staining intensity for mock-transfected cells. **(E)** Effect of PJ constructs on PM recruitment of PI4P/PI(4,5)P₂-binding green fluorescent protein (GFP)-PH-Osh2x2 (example images) and the PI(4,5)P₂-selective PH-PLC δ 1 and Tubby_c domains (means $\pm$ SEM of 10 to 18 cells).



PI(4,5)P₂-biosensors such as the PLCδ1 PH (PH-PLCδ1) or Tubby C-terminal (Tubby_c) domains (17) (Fig. 1E and fig. S2).

Fig. 2. Dependence of clathrin-mediated endocytosis, PI3K, and PLC signaling on PM PI(4,5)P₂, but not PI4P. **(A)** Effect of PM-recruited PJ constructs (red) on the uptake of transferrin (AlexaFluor488, 20 μg/ml, green) over 15 min before the removal of surface bound transferrin with a pH 2.5 wash. The bar chart shows the proportion of cells showing uptake of transferrin-associated fluorescence. Data are means ± SEM (*n* = 4). **(B)** Effect of PM recruitment of PJ constructs on the PI(3,4)P₂/PI(3,4,5)P₃ reporter GFP-PH-Akt after stimulation of serum-starved cells for 2 min with insulin-like growth factor 1 (IGF-1). Data are means ± SEM from 15 to 26 cells. **(C)** Effect of PM-recruited PJ constructs on PLC-mediated Ca²⁺ signals (monitored with Ca²⁺ indicator Fluo4-AM) after stimulation of endogenous P2Y₂-receptors with 100 μM adenosine triphosphate in either calcium-free (100 μM EGTA) or Ca²⁺-containing (1.8 mM) medium. Data are means ± SEM from 11 to 26 cells.

To deplete PI4P specifically, we inactivated PJ's INPPE domain by mutation, making a chimera we call PJ-Sac. Recruitment of this enzyme

to the PM caused depletion of PM PI4P staining but had no effect on PM PI(4,5)P₂ staining (Fig. 1D) or localization of PH-Osh2x2, PH-PLCδ1,

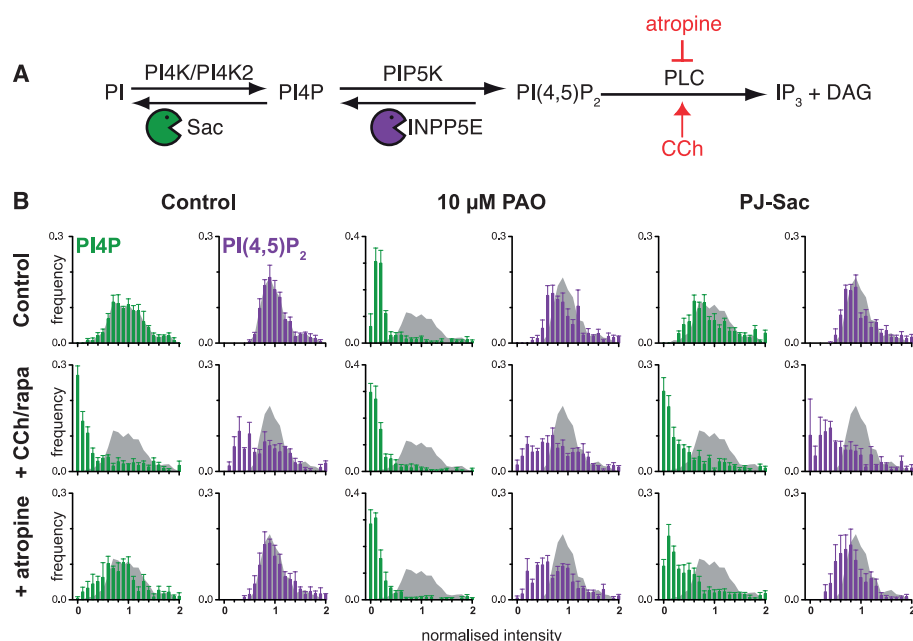
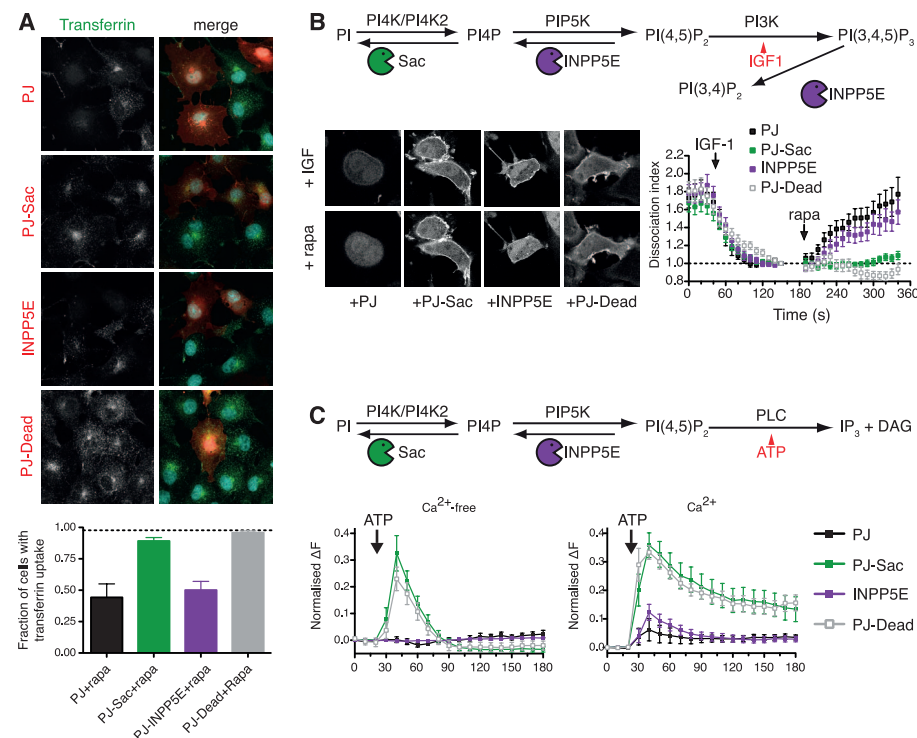
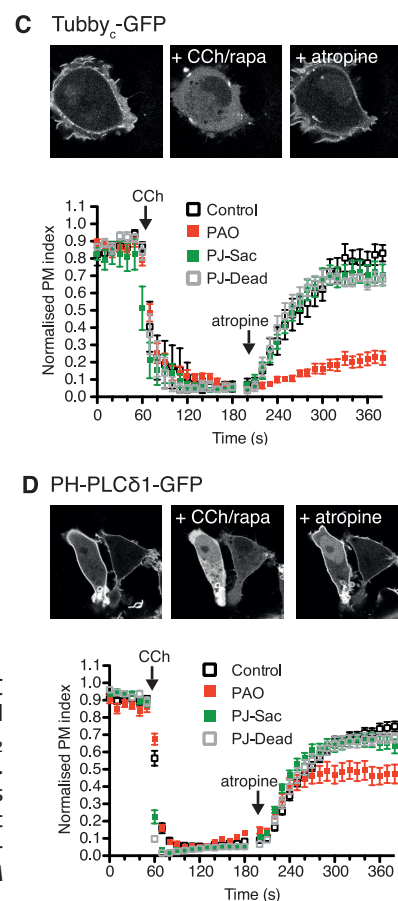


Fig. 3. Requirements of PI4K activity, but not PM PI4P, for resynthesis of PI(4,5)P₂ after robust PLC activation. **(A)** In muscarinic M1 receptor-expressing cells, PLC activity is stimulated with carbachol (CCh) and inactivated by atropine. **(B)** Effect of PAO or PJ-Sac (recruited to the PM with rapamycin) on PI4P and PI(4,5)P₂ staining before and after 2 min 1 mM CCh stimulation or after a further 3 min of 10 μM atropine treatment. Histograms are the relative frequency of occurrence of PI4P and PI(4,5)P₂ staining intensities expressed as means ± SEM (*n* = 4). Gray peaks are data from control, unstimulated cells. **(C and D)** Effect of PAO or PJ-Sac recruited to the PM on PI(4,5)P₂ resynthesis assayed with the PI(4,5)P₂ biosensors Tubby_c-GFP (C) or PH-PLCδ1-GFP (D) during stimulation with CCh and subsequent inhibition with atropine. Data are means ± SEM of 8 to 26 cells. Images show cells coexpressing the reporters with Lyn₁₁-FRB-CFP and PJ-Sac.



or Tubby_c (Fig. 1E and fig. S2). In fact, cells showing the largest degree of PI4P depletion induced by LY294002, PAO, or PJ-Sac had scarcely altered PI(4,5)P₂ abundance (fig. S1, C and D). The effects of the chimeras depended on rapamycin-induced membrane recruitment (fig. S1B) and were not observed with PJ-Dead, a chimera with inactivated sac and INPP5E domains (fig. S1B). PJ did not affect Golgi PI4P or endosomal PI3P staining (fig. S3).

These observations demonstrate that most PM PI4P is not required to maintain the steady-state PI(4,5)P₂ pool. However, PI4P may still act as a reserve for cellular functions associated with continued consumption, and therefore replenishment, of PM PI(4,5)P₂. Such processes include clathrin-mediated endocytosis of transferrin (18), continued generation of the lipid second messengers PI(3,4,5)P₃ and PI(3,4)P₂, and generation of Ca²⁺-mobilizing inositol 1,4,5-trisphosphate (IP₃).

Indeed, PM recruitment of PJ or INPP5E inhibited all of these processes (Fig. 2, A, B, and C; and fig. S4). Depletion of PM PI4P with PJ-Sac, on the other hand, had no effect (Fig. 2 and fig. S4), and thus, PI4P is dispensable for maintaining the functionally relevant PI(4,5)P₂ pool.

One possible explanation for this lack of effect is that a small fraction of the total PM PI(4,5)P₂ pool is consumed during endocytosis or signaling. In contrast, activation of PLC by muscarinic M1 (8, 19) or angiotensin II receptors (7) leads to consumption of up to 90% of PI(4,5)P₂. We therefore used transient overexpression of M1 receptors in COS-7 cells to investigate resynthesis of PI(4,5)P₂ (Fig. 3A). Stimulation of M1-expressing cells led to reduced PI(4,5)P₂ and PI4P staining, which returned to prestimulation levels after addition of the M1 receptor antagonist atropine (Fig. 3B). PM-recruited PJ-Sac had no effect on this recovery of PI(4,5)P₂ staining, despite sustained depletion of PM PI4P (Fig. 3B and fig. S5). Likewise, PI(4,5)P₂ biosensors showed translocation from the PM upon PLC activation, but their return to the PM after atropine addition was unaffected by PJ-Sac recruitment (Fig. 3, C and D, and fig. S6).

These data indicate that PM PI4P seems to be redundant for synthesis of PI(4,5)P₂. Intuitively, such a result seems contradictory, given the known requirements for PI4K in this pathway. Indeed, the PI4K inhibitor PAO prevented resynthesis of PI(4,5)P₂ assayed with PI(4,5)P₂ staining (Fig. 3B and fig. S5) or the Tubby_c and PH-PLCδ1 reporters (Fig. 3, C and D) (7, 10). These experiments show that, despite a requirement for PI4K, PI(4,5)P₂ production continues in the absence of PM PI4P, either because of the efficiency of PIP5K in consuming residual PI4P [i.e., the PI4P used for PI(4,5)P₂ synthesis is synthesized ad hoc by PI4Ks], or else PI4P is supplied from other membranes (20). Either way, we conclude that the majority of PM PI4P is not required for PI(4,5)P₂ synthesis.

If PM PI(4,5)P₂ and its functions are independent of PM PI4P, why do cells maintain substantial quantities of PI4P? Many proteins selectively target the PM through basic amino acid stretches that interact with anionic lipid head groups (3, 21); monovalent lipids such as PI and phosphatidylserine (PS) are present at high concentrations in several membranes (22), whereas an abundance of polyanionic inositol lipids is unique to the PM (13, 22). These polyanionic lipids concentrate around stretches of polybasic residues through nonspecific electrostatic interactions, increasing binding affinity (3). We therefore reasoned that PI4P might contribute to this electrostatic interaction. We screened the localization of short peptide sequences from PM proteins before and after depletion of PI4P and/or PI(4,5)P₂ (Fig. 4A and fig. S7). These included amphipathic peptides, such as the myristoylated alanine-rich C-kinase substrate effector domain (MARCKS-ED) and Rit1 guanosine triphosphatase C terminus (Rit1-tail), and lipid-anchored polybasic sequences, such as the C terminus of K-Ras (K-Ras

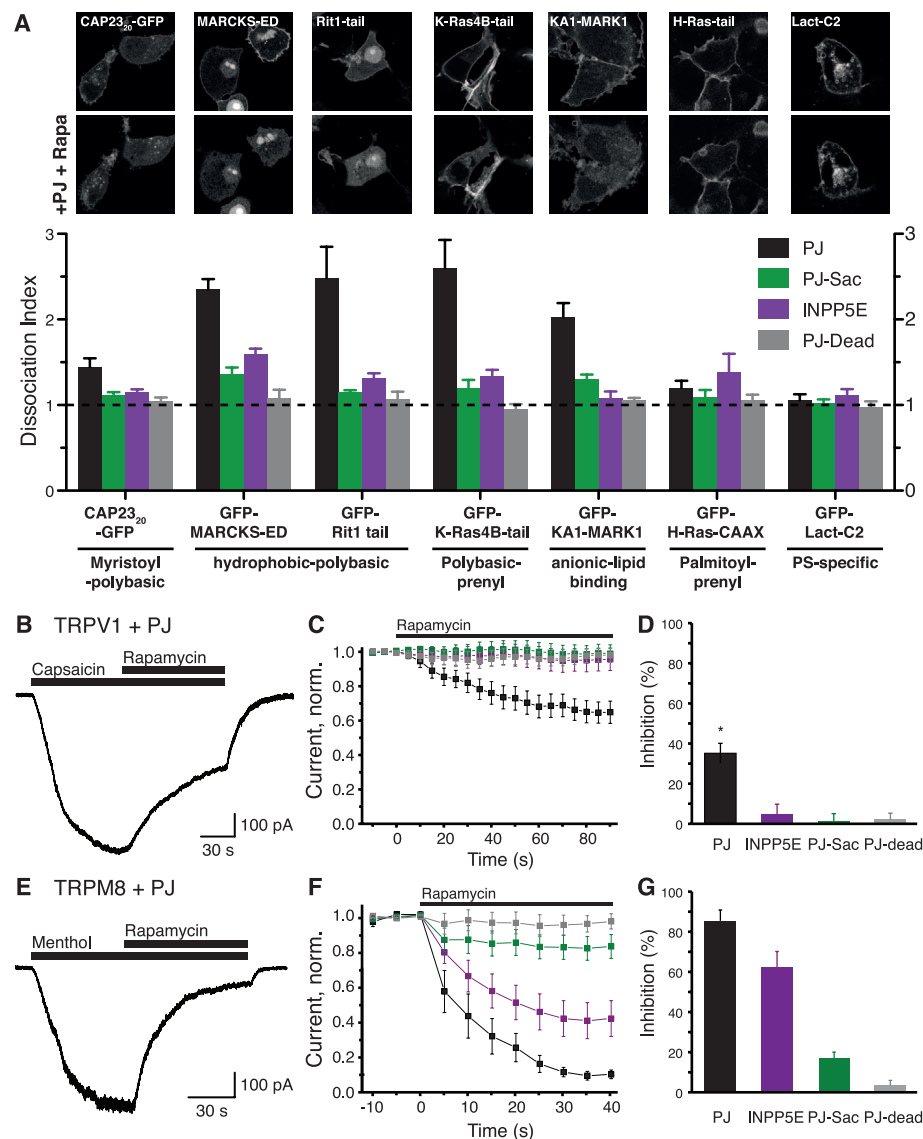


Fig. 4. (A) Targeting of proteins with polybasic motifs to the PM by PI4P and PI(4,5)P₂. Representative images before and after rapamycin treatment, and dissociation index of cells transfected with the indicated GFP-tagged motifs, Lyn₁₁-FRB-CFP and the indicated PJ construct. Lact-C2, lactadherin C2 domain. Data are means $\pm$ SEM for 8 to 19 cells. (B to D) Capsaicin-induced currents in human embryonic kidney (HEK) 293 cells expressing TRPV1 are enabled by the presence of either PI4P or PI(4,5)P₂. (B) Specimen showing rapamycin-induced inactivation in the presence of PJ. (C) Time course of the capsaicin-induced current in the presence of PJ, INPP5E, PJ-Sac, or PJ-Dead. (D) Percentage of inhibition at the end of the 90-s coapplication of rapamycin (1 μ M) (n = 8). (E to G) Menthol-induced currents in HEK293 cells expressing TRPM8 depend primarily on the presence of PI(4,5)P₂. (E) Representative specimen showing the effect of PJ. (F) Time course of the menthol-induced current in the presence of PJ, INPP5E, PJ-Sac, or PJ-Dead. (G) Percentage of inhibition at the end of the 90-s coapplication of rapamycin (1 μ M) (n = 7).

tail) and the N terminus of cortical cytoskeleton-associated protein of ~23 kD (CAP23₂₀). We also assayed the kinase-associated 1 (KA1) domain from microtubule-associated protein–microtubule affinity–regulating kinase 1 (MARK1), which interacts nonspecifically with acidic lipids (23). In all cases, combined removal of PI4P and PI(4,5)P₂ caused depletion of the proteins from the PM (Fig. 4A and fig. S7), with little effect when either lipid was depleted alone (13). Proteins that retained a secondary membrane targeting motif, such as prenylated K-Ras tail, were still found in the PM but were no longer enriched there compared with the amounts in other [presumably negatively charged (22)] membranes (Fig. 4A and fig. S8A). These effects were due to nonspecific electrostatic interactions, because no effect was seen on the PS-specific lactadherin C2 domain (22) or the C terminus of H-Ras, which interacts with the membrane solely through its hydrophobic lipid moieties (Fig. 4A and fig. S7). Measuring K-Ras tail's PM dissociation rate by fluorescence recovery after photobleaching (24) after PI4P and/or PI(4,5)P₂ depletion revealed that the two lipids made similar contributions to the protein's electrostatic interactions with the PM in vivo (fig. S8).

PI(4,5)P₂ has been proposed to be a molecular switch that restricts the activity of several ion channels to the PM (25), a phenomenon that can be highly specific for PI(4,5)P₂ (1, 26–28). We wondered whether this is typical for all channels or whether some have a more general polyanionic lipid requirement, which can also be fulfilled by PI4P. For example, the heat and capsaicin-activated transient receptor potential vanilloid 1 (TRPV1) cation channel can be both inhibited and activated by PI(4,5)P₂ and possibly PI4P (29). Translocation of PJ-Sac or INPP5E had no effect on prolonged (Fig. 4, B to D) or repetitive (fig. S9) capsaicin activation of TRPV1, but it was inhibited when both PI4P and PI(4,5)P₂ were depleted by PJ (Fig. 4, B to D, and fig. S9). Therefore, it appears that either lipid is sufficient for TRPV1 channel activity. However, this does not apply to all lipid-activated cation channels. For example, the menthol-activated transient receptor potential melastatin 8 (TRPM8) channel is specifically dependent on PI(4,5)P₂ (12) and was inhibited by PI(4,5)P₂ depletion, but not by removing PI4P with PJ-Sac (Fig. 4, E to G).

Our results reveal an unanticipated role for PI4P at the PM of cells: Most of it is not required to support synthesis of PI(4,5)P₂. Rather, PI4P makes an autonomous contribution to the polyanionic lipid pool that defines the inner leaflet of the PM, a function it shares with PI(4,5)P₂. We suggest that PI4P fulfills the need of any PM functions that simply require polyvalent anionic lipids. This leaves PI(4,5)P₂ free to undergo rapid turnover and regulate its large repertoire of specific effector proteins, which may decrease its effective free concentration, without deleteriously perturbing the unique and defining electrostatic properties of the PM.

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Supplementary Materials

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Lineage Tracing Reveals Lgr5⁺ Stem Cell Activity in Mouse Intestinal Adenomas

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The concept that tumors are maintained by dedicated stem cells, the so-called cancer stem cell hypothesis, has attracted great interest but remains controversial. Studying mouse models, we provide direct, functional evidence for the presence of stem cell activity within primary intestinal adenomas, a precursor to intestinal cancer. By “lineage tracing” using the multicolor Cre-reporter *R26R-Confetti*, we demonstrate that the crypt stem cell marker Lgr5 (leucine-rich repeat–containing heterotrimeric guanine nucleotide–binding protein–coupled receptor 5) also marks a subpopulation of adenoma cells that fuel the growth of established intestinal adenomas. These Lgr5⁺ cells, which represent about 5 to 10% of the cells in the adenomas, generate additional Lgr5⁺ cells as well as all other adenoma cell types. The Lgr5⁺ cells are intermingled with Paneth cells near the adenoma base, a pattern reminiscent of the architecture of the normal crypt niche.

Intestinal tumorigenesis is thought to result from sequentially acquired mutations in specific genes, driving progression from premalignant

precursor lesions called adenomas to invasive malignancies (1). Adenomas are formed by mutational activation of the Wnt signaling pathway, most notably by loss of the *APC* (adenomatous polyposis coli) tumor suppressor gene (2). By using the knock-in allele *Lgr5^{EGFP-Ires-CreERT2}*, we have shown that the cell surface receptor Lgr5 (leucine-rich repeat–containing heterotrimeric guanine nucleotide–binding protein–coupled receptor 5) marks normal tissue stem cells in stomach, small intestine, colon, and hair follicles (3). This allele allows visualization of Lgr5⁺ stem cells by GFP (green fluorescent protein). Moreover,

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the coexpressed Cre fusion protein can be activated at will in these stem cells by tamoxifen injection and will then recombine two adjacent LoxP sites. When a so-called Cre reporter allele is crossed into the *Lgr5* knock-in mouse strain, tamoxifen injection can result in a visible change (e.g., a color) in an individual *Lgr5*⁺ cell that is inherited by all of its daughter cells. This strategy to document the behavior of stem cells is called lineage tracing. Although deletion of *Apc* in other cells of the intestinal epithelium does not lead to adenoma formation, we have shown that *Apc*-mutant *Lgr5*⁺ stem cells form progressively growing adenomas (4). Comparable adenomas were obtained by using another Cre driver (i.e., *CD133*^{CreERT2}) to induce *Apc* gene deletion in these same stem cells (5). We noted that macroscopic adenomas in these mice contained low numbers of *Lgr5*⁺ cells, suggesting the preservation of a stem cell hierarchy within the tumors (4).

To investigate the existence of such a cellular hierarchy in adenomas, we exploited the multicolor Cre reporter termed *R26R-Confetti* (6). When *R26R-Confetti* is crossed into the *Lgr5* knock-in mouse strain, tamoxifen injection will allow single *Lgr5*⁺ stem cells to randomly adopt one of the four fluorescent colors encoded in the *R26R-Confetti* allele. Of importance for the current study, two of the four colors remain in the *R26R-Confetti* allele after tamoxifen injection (blue with red or green with yellow). One of these is active, the other silent (Fig. 1A). In theory, a second tamoxifen injection can induce “flipping” from the active color to the silent color (here termed retracing). To test this, we globally activated *R26R-Confetti* in the intestinal epithelium by using the β -naphthoflavone-inducible *Ah-Cre* mouse strain (7). As hypothesized, the already rearranged *R26R-Confetti* reporter could be flipped to the remaining color by a second tamoxifen

injection (fig. S1). Spontaneous color conversions were never observed.

To study the behavior of *Lgr5*⁺ cells within *Apc*-mutant adenomas, we then crossed *Lgr5*^{EGFP-Ires-CreERT2}/*Apc*^{fl/fl} mice with the *R26R-Confetti* strain. Upon stochastic Cre induction by low-dose tamoxifen in adult mice, intestinal adenomas developed from *Apc*-deleted *Lgr5*⁺ stem cells. After 4 weeks, we observed large adenomas (fig. S2, A to C) that either uniformly expressed a single *Confetti* color or consisted of anatomically separate segments that were each marked uniformly in a single *Confetti* color (Fig. 1B). The latter result indicates that such large adenomas were derived from several independent *Apc*-mutant stem cells, most likely located in adjacent crypts. Indeed, such segmental oligo-clonality of adenomas has been described (8, 9). Of note, recombination of the floxed *Apc* allele was more efficient than the activation of *R26R-Confetti* in

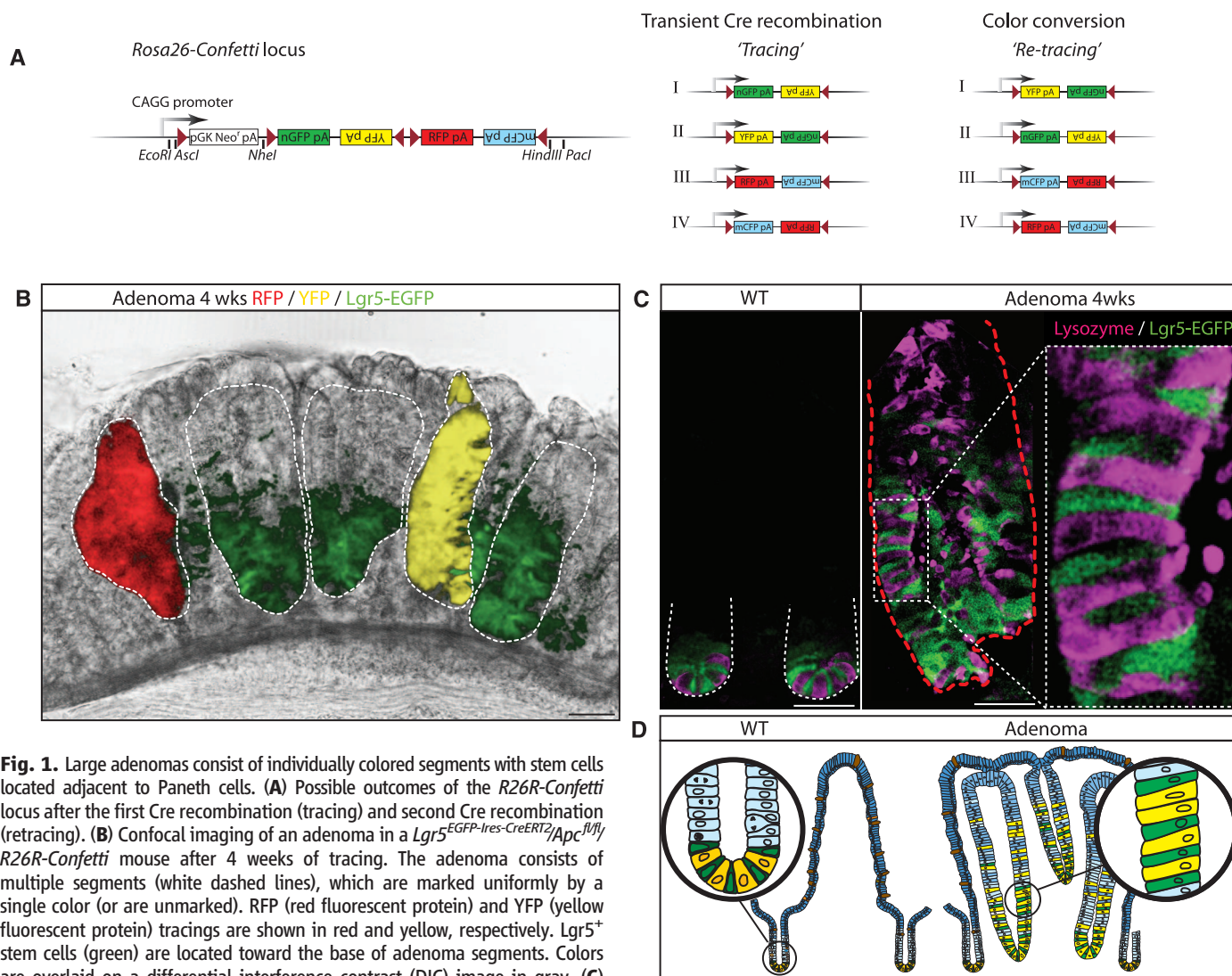


Fig. 1. Large adenomas consist of individually colored segments with stem cells located adjacent to Paneth cells. (A) Possible outcomes of the *R26R-Confetti* locus after the first Cre recombination (tracing) and second Cre recombination (retracing). (B) Confocal imaging of an adenoma in a *Lgr5*^{EGFP-Ires-CreERT2}/*Apc*^{fl/fl} *R26R-Confetti* mouse after 4 weeks of tracing. The adenoma consists of multiple segments (white dashed lines), which are marked uniformly by a single color (or are unmarked). RFP (red fluorescent protein) and YFP (yellow fluorescent protein) tracings are shown in red and yellow, respectively. *Lgr5*⁺ stem cells (green) are located toward the base of adenoma segments. Colors are overlaid on a differential interference contrast (DIC) image in gray. (C) Distribution of stem cells marked by *Lgr5*-GFP and Paneth cells, marked by lysozyme (purple) at the base of wild-type (WT) crypts (left). (Right) *Lgr5*⁺ stem cells (green) are located adjacent to Paneth cells (purple) toward the base of

an adenoma segment (indicated by the red dashed line). (D) Schematic representation of the intermingled stem cells and Paneth cells in wild-type crypts (left) and adenomas (right). Scale bars indicate 50 μ m.

the same stem cell, because not all adenomas or adenoma segments expressed a *Confetti* color.

In normal small intestinal crypts, stem cells are always adjacent to Paneth cells. These Paneth cells serve as niche cells to the stem cells by producing signaling molecules such as Wnt, epidermal growth factor (EGF), and Notch ligands (10). The *Apc*-mutant adenomas contained Paneth cells (Fig. 1C) but typically lacked mature goblet cells and enterocytes (fig. S2, D and E). *Lgr5*⁺ adenoma cells were often located adjacent to adenoma Paneth cells, suggesting the existence of an adenoma stem cell niche (Fig. 1C). Indeed, these clusters of *Lgr5*⁺ cells and Paneth cells tended to be located toward the base of the wedge-shaped adenoma segments, a situation reminiscent of normal crypt architecture (Fig. 1D). We noted a similar arrangement in colon adenomas, in which areas of *Lgr5*⁺ cells coincided with the location of the colon-counterpart of the Paneth cell, the deep crypt secretory cell, marked by *Reg4* (10, 11) (fig. S3). To mimic the next step in the tumorigenic process, we additionally crossed in an allele of *K-ras* that can be oncogenically activated by Cre (*K-ras*^{LSL-G12D}) (12). We observed

a dramatic acceleration of the growth of these *Apc/Kras* mutant tumors compared with *Apc*-mutant adenomas. The mice became moribund within 7 to 10 days, yet the cryptlike architecture was preserved in the *Apc/Kras* double-mutant tumors (fig. S4).

Next, we set out to determine the transcriptional profile of *Lgr5*-GFP^{hi} cells in adenomas to allow comparison to normal crypt *Lgr5*-GFP^{hi} stem cells. We sorted *Lgr5*-GFP^{hi} and *Lgr5*-GFP^{lo} cells from 30-day-old adenomas and performed comparative gene expression analyses of the two populations. Gene set enrichment analyses demonstrated that the *Lgr5*-GFP^{hi} stem cell signature determined for normal crypts (13) was strongly enriched in the *Lgr5*-GFP^{hi} adenoma cells (Fig. 2). This observation suggested that the *Lgr5*-GFP^{hi} adenoma cells retain stem cell properties. To substantiate this notion, we compared the clonogenic potential of *Lgr5*-GFP^{hi} and *Lgr5*-GFP^{lo} adenoma cells in a defined culture system developed for normal tissue *Lgr5*-GFP^{hi} stem cells (14, 15). In this assay, normal *Lgr5*-GFP^{hi} cells grow out into crypt-villus organoids, whereas *Lgr5*-GFP^{lo} rarely grow out, consistent with the idea that *Lgr5*-GFP^{hi}

cells constitute multipotent stem cells. Analysis of the clonogenic potential of *Lgr5*-GFP^{hi} and *Lgr5*-GFP^{lo} adenoma cells in this culture system recapitulated the observations made in normal tissue: The colony-forming efficiency of *Lgr5*-GFP^{hi} adenoma cells was about 20 times greater than that of *Lgr5*-GFP^{lo} adenoma cells (Fig. 2, D and E).

To trace the fate of *Lgr5*⁺ adenoma cells in vivo, we injected 10-week-old *Lgr5*^{EGFP-Ires-CreERT2}/*Apc*^{fl/fl}/*R26R-Confetti* mice with tamoxifen. After allowing *Confetti*-marked adenomas to develop for 24 days, we induced lineage retracing by a second tamoxifen injection. Adenomas were analyzed by confocal microscopy at different time points after the second Cre pulse. We observed rare retracings within large adenomas (~six color retracings per 100 adenoma segments), which invariably involved switching to the silent color in that particular adenoma. As an example, rare blue cells were observed within red adenomas early after lineage tracing (Fig. 3). Because we observed retracing events in only ~6% of *Confetti*-marked adenomas and these presented typically as a single cell at one day after the second tamoxifen

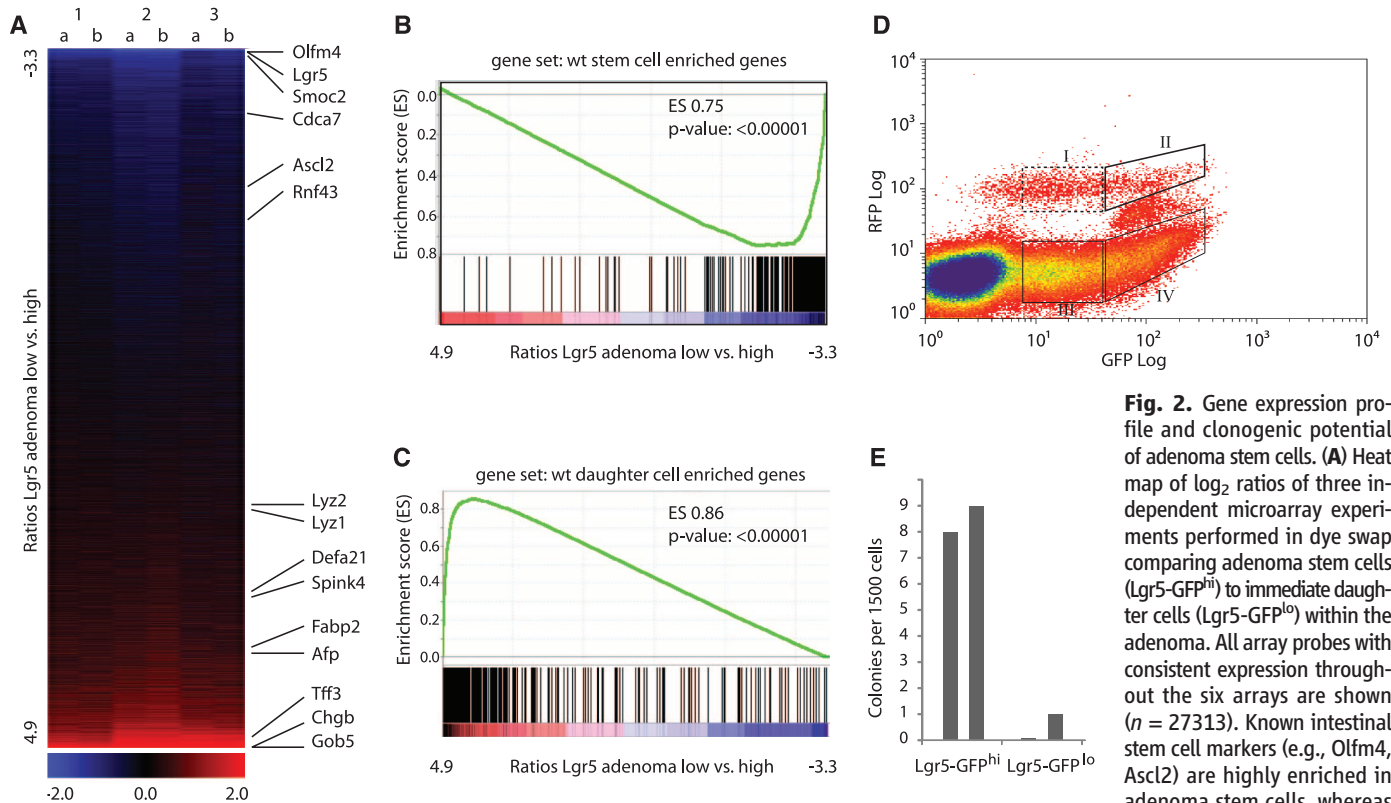


Fig. 2. Gene expression profile and clonogenic potential of adenoma stem cells. (A) Heat map of log₂ ratios of three independent microarray experiments performed in dye swap comparing adenoma stem cells (*Lgr5*-GFP^{hi}) to immediate daughter cells (*Lgr5*-GFP^{lo}) within the adenoma. All array probes with consistent expression throughout the six arrays are shown (*n* = 27313). Known intestinal stem cell markers (e.g., *Olfm4*, *Ascl2*) are highly enriched in adenoma stem cells, whereas their daughter cells express markers for the four cell lineages in the gut: goblet cells (*Gob5*, *Tff3*), enteroendocrine cells (*Chgb*), enterocytes (*Fabp2*, *Afp*), and Paneth cells (*Lyz1/2*, *Dfa21*). (B) Gene set enrichment analysis (GSEA). Genes are ranked according to their differential expression between *Lgr5*-GFP^{lo} cells and *Lgr5*-GFP^{hi} cells. Black bars below the graph depict the position of 291 genes significantly enriched in normal *Lgr5*⁺ intestinal stem cells [GEO data set GSE23672 (13)]. A highly significant enrichment of this gene set was detected toward the genes highly expressed in *Lgr5*-GFP^{hi} adenoma cells. (C) GSEA analysis on the same data set as in (B), now analyzed for the top 500 genes significantly enriched in daughter cells of normal *Lgr5*⁺ stem cells. A highly significant enrichment of this gene set was detected toward the genes with higher expression in *Lgr5*-GFP^{lo} adenoma cells. ES, enrichment score. (D) Fluorescence-activated cell sorting profile of *Lgr5*-EGFP-Ires-CreERT2/*Apc*^{fl/fl}/*R26R-Confetti* mice 7 days after induction. Cells positive for RFP are adenoma cells. Gates I and II represent the *Lgr5*-GFP^{lo} and *Lgr5*-GFP^{hi} adenoma cells, respectively. Gates III and IV represent WT *Lgr5*-GFP^{lo} and *Lgr5*-GFP^{hi} cells. (E) Colony-forming efficiency of sorted adenoma cells. The outgrowth efficiency of *Lgr5*-GFP^{hi} adenoma cells is about 20 times higher than that of *Lgr5*-GFP^{lo} adenoma cells.

injection (Fig. 3, D and E), we considered these to represent clonal retracing events. In other words, these cells were derived from a single adenoma stem cell. As expected, the blue cells were mostly located near the base of the wedge-shaped adenoma segments, the location of the *Lgr5*⁺ cells.

No spontaneous color conversion was observed in control mice that were not given a second Cre pulse, in a total of 107 adenomas analyzed 27 days after one pulse of tamoxifen.

Six days after retracing, the number of blue cells within red adenomas had increased. More-

over, the growing blue clones presented as ribbonlike structures emanating from the base of the segments and projecting toward to the intestinal lumen (Fig. 3B), thus resembling, in a crude and somewhat chaotic way, the more-organized tracing patterns observed in healthy intestinal tissue

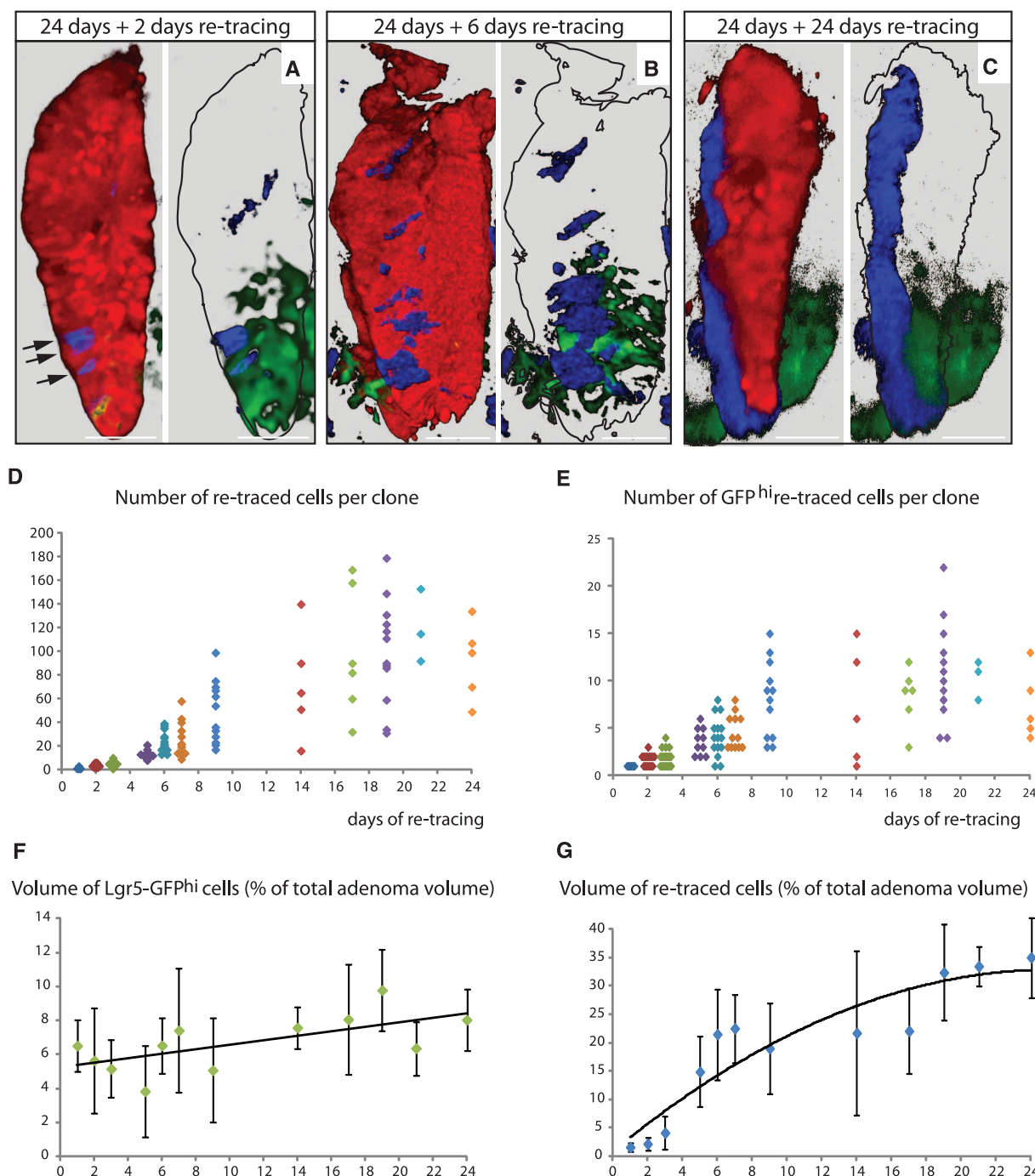
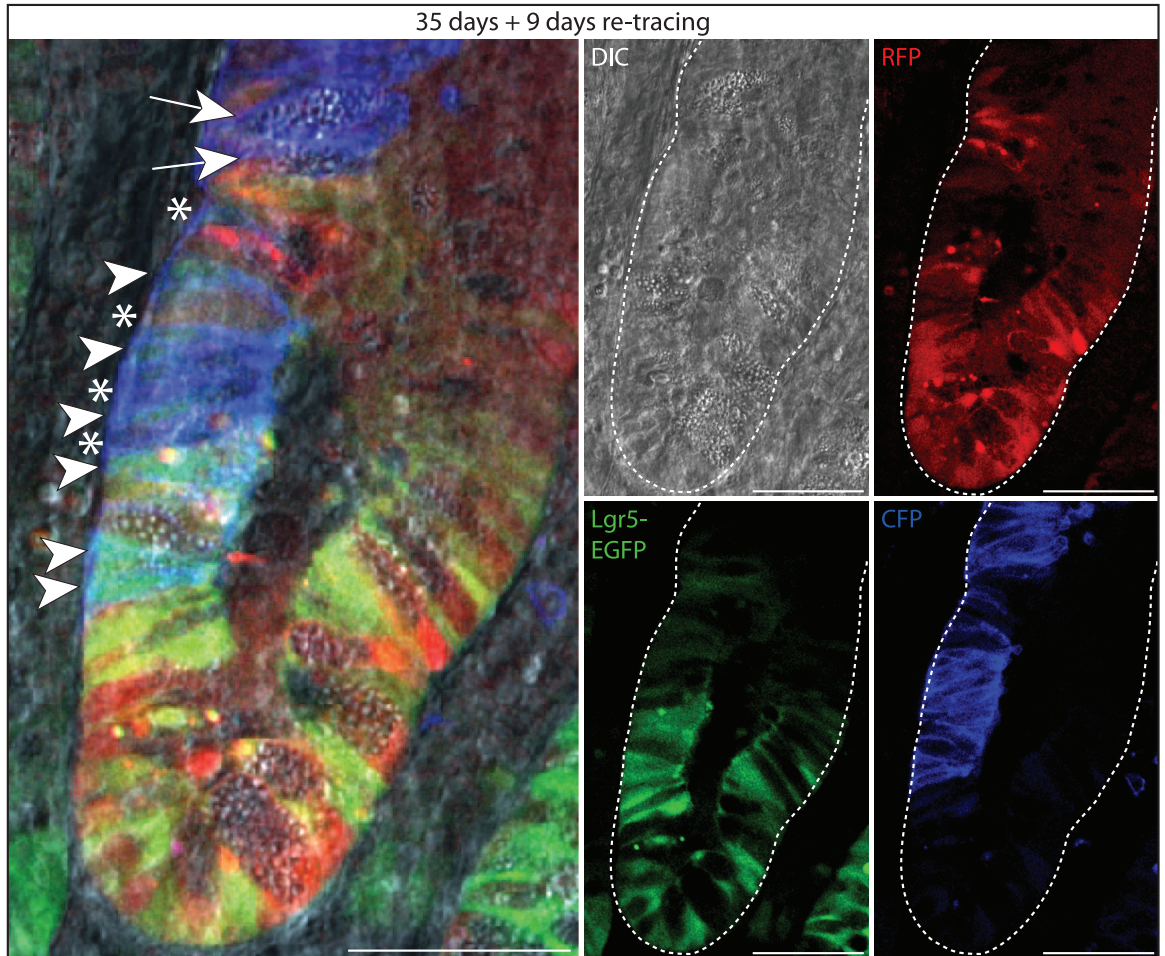


Fig. 3. Retracing of *Lgr5*⁺ adenoma cells. (A) *Apc*-deficient adenomas were allowed to develop for 24 days in *Lgr5-EGFP-Ires-CreERT2/Apc^{fl/y}/R26R-Confetti* mice before retracing of *Lgr5*⁺ adenoma cells was induced. Two days after retracing, CFP [cyan fluorescent protein (blue)]-positive cells (arrows) were observed at the bottom of a red adenoma segment. *Lgr5-EGFP* is shown in green. (B) Six days after retracing, blue ribbons of cells were present within red adenoma segments. (C) Twenty-four days after retracing, blue cells comprised

a large part of the adenoma. (D and E) Quantification of the total amounts of re-traced cells (D) and the amounts of *Lgr5*-GFP^{hi} re-traced cells (E) over time. (F) Calculated volume of GFP^{hi} cells as a percentage of the total volume of the adenoma segment. Error bars represent SEM. A linear trend line is given, $R^2 = 0.41$. (G) Calculated volume of the re-traced cells as a percentage of the total volume of the adenoma segment. Error bars represent SEM. A second-order polynomial trend line is given, $R^2 = 0.86$.

Fig. 4. $Lgr5^+$ adenoma cells expand and give rise to adenoma Paneth cells. A red adenoma segment retraced for 9 days after 35 days of adenoma development in a $Lgr5$ -EGFP-Ires-CreERT2/ $Apc^{fl/fl}/R26R$ -Confetti mouse. The left image is an overlay of four channels; DIC is shown in gray, the original segment in red, $Lgr5^+$ cells in green, and the retraced cells in blue. Within the red segment, a blue clone appears, originating at the $Lgr5$ -EGFP $^+$ base of the cryptlike structure. Multiple $Lgr5$ -GFP $^+$ adenoma cells occur within the clonal ribbon (arrowhead), demonstrating the expansion of $Lgr5^+$ adenoma cells. In addition, at least two blue Paneth cells (arrows) and multiple blue $Lgr5^-$ cells, resembling transit-amplifying cells (asterisks), are present. Scale bars, 50 μ m.



(3). From day 9 to day 24 after retracing, the blue cells expanded to compose up to 30% of the volume of the adenomas (Fig. 3, C and G). A quantification of clone size over time is given in Fig. 3D. The number of $Lgr5$ -GFP hi stem cells within the retraced population also increased over time, indicating that $Lgr5^+$ adenoma stem cells clonally expanded within established adenomas (Fig. 3E). The total number of $Lgr5$ -GFP hi cells comprised a minority of the adenoma cells. We found that around 5 to 10% of the adenoma cells were $Lgr5$ -GFP hi (Fig. 3F). This number is similar to that of normal crypts, which consist of 150 to 200 cells and harbor about 15 stem cells each, thus reinforcing the notion that adenomas retain characteristics of the crypt stem cell niche.

To address whether $Lgr5$ -GFP hi adenoma cells are able to generate all cell types of the adenoma, we studied the phenotype of individual cells within the clonal offspring of an $Lgr5^+$ adenoma cell. As an example, Fig. 4 depicts a red adenoma after 35 days of tracing, with a subsequent retracing for 9 days, in which an $Lgr5^+$ adenoma cell gave rise to a blue ribbonlike clone (see also fig. S5). Multiple $Lgr5$ -GFP $^+$ adenoma cells were observed within the clonal ribbon (arrowheads), demonstrating the production of additional $Lgr5^+$ adenoma cells within the ribbon. In addition, at least two blue Paneth cells

(arrows) and multiple blue $Lgr5^-$ cells, resembling transit-amplifying cells (asterisks), could be observed after 9 days of retracing within the clonal ribbonlike structure. These results indicate that $Lgr5^+$ adenoma cells give rise to the other cell types in the adenoma. Combined with the observation that large parts of an adenoma can emerge from a single $Lgr5^+$ adenoma cell, these observations qualify these cells as the multipotent stem cells of the adenoma.

The original studies that propose the existence of cancer stem cells in human colorectal cancer have their basis in transplantation of sorted cell populations into immunodeficient mice (16–18). The manipulations required for sorting, combined with the xenotransplantation setting, impose inherent limitations to the interpretation of the outcome of this assay (19). In an alternative approach, single-cell sorting of cultured primary colorectal cancers has been used to demonstrate that about 1 in 20 cells has the capacity in vitro of multilineage differentiation, considered an attribute of stem cells (20). In a follow-up study, this culture-based assay was combined with the classic xenograft approach to demonstrate that colon cancer stem cells display high Wnt pathway activity (21). The stem cell marker used in the current study, $Lgr5$, is encoded by a Wnt target gene and itself constitutes a Wnt receptor component (22).

Battle and colleagues used markers enriched in normal colon $Lgr5^+$ stem cells to visualize a stemlike cell population in human colon cancers, residing at the base of structures that resemble normal crypts (21). Indeed, the presence of $Lgr5^+$ stem cells and of all differentiated lineages within colon cancers was confirmed by single-cell polymerase chain reaction (23). These latter two studies imply that our current functional observations on $Lgr5^+$ stem cells in mouse adenomas extend to human colon carcinomas. Although adenomas represent only the first stage of intestinal tumorigenesis, the composition of these tumors, as revealed in this study, is fully compatible with the original notion of Pierce and Speers (24) that “carcinomas are caricatures of tissue renewal, in that they are composed of a mixture of malignant stem cells, which have a marked capacity for proliferation and a limited capacity for differentiation under normal homeostatic conditions, and of the differentiated, possibly benign, progeny of these malignant cells.”

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Supplementary Materials

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Materials and Methods
Figs. S1 to S5
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Closed-Loop Control of Epilepsy by Transcranial Electrical Stimulation

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Many neurological and psychiatric diseases are associated with clinically detectable, altered brain dynamics. The aberrant brain activity, in principle, can be restored through electrical stimulation. In epilepsies, abnormal patterns emerge intermittently, and therefore, a closed-loop feedback brain control that leaves other aspects of brain functions unaffected is desirable. Here, we demonstrate that seizure-triggered, feedback transcranial electrical stimulation (TES) can dramatically reduce spike-and-wave episodes in a rodent model of generalized epilepsy. Closed-loop TES can be an effective clinical tool to reduce pathological brain patterns in drug-resistant patients.

A successful, although not well-understood, therapy in drug-resistant cases of Parkinson's disease and depression is deep brain stimulation (1–3), in which high-frequency stimulation is applied continuously. In many diseases,

such as epilepsies, events recur unpredictably and often are separated by long interictal intervals (4–6). In such instances, a closed-loop, transient feedback control could abort seizure episodes without inducing detrimental side effects of

continuous stimulation (7–13). We attempted to achieve seizure control by means of closed-loop transcranial electrical stimulation (TES) in a rodent model of generalized (“petit mal”) epilepsy (14, 15) because previous experiments have shown that even very weak TES can reliably entrain neurons in widespread cortical areas (16–20).

We first demonstrated the effect of TES on cortical excitability. Local field potentials (LFPs) and multiple-unit activity (MUA) were recorded by chronically implanted tripolar electrodes (Fig. 1A) and placed in the deep and superficial layers of the frontal and parietal cortical areas (21). TES was applied either between the left and right

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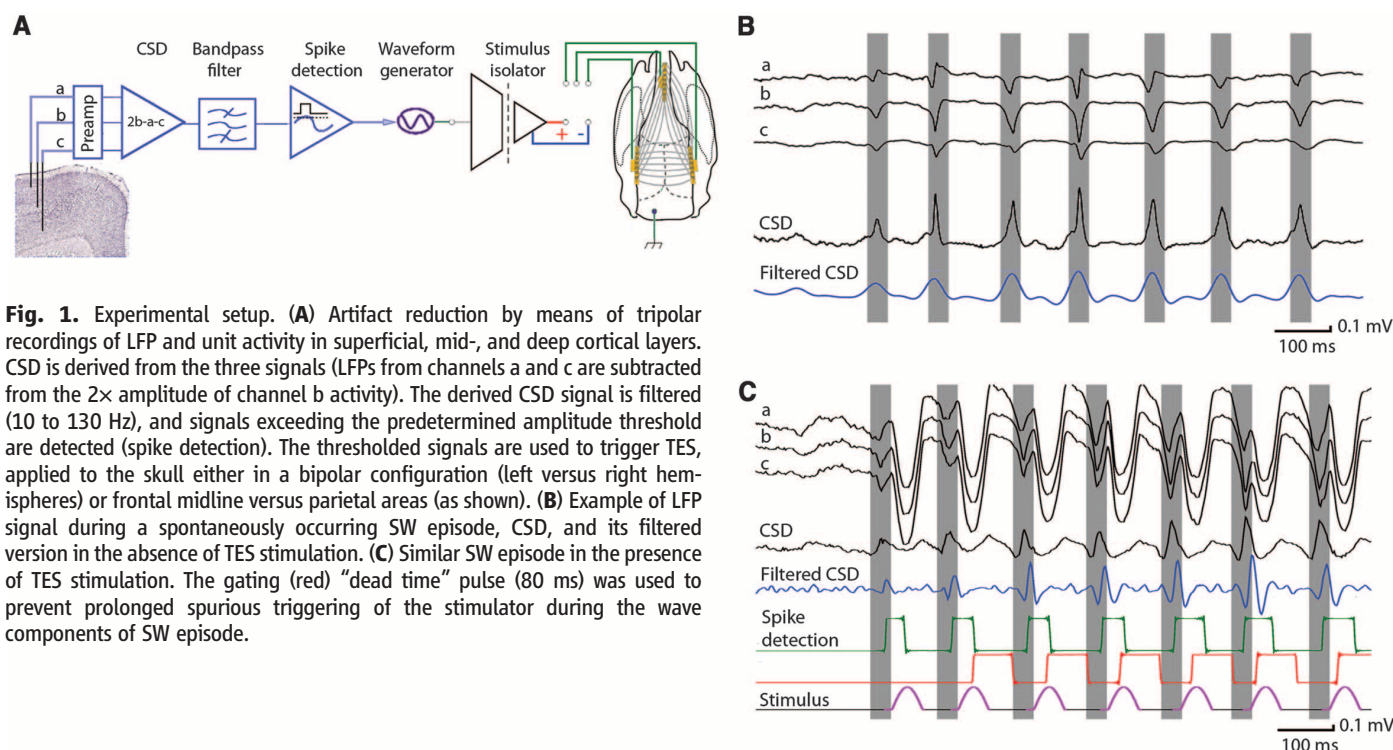


Fig. 1. Experimental setup. (A) Artifact reduction by means of tripolar recordings of LFP and unit activity in superficial, mid-, and deep cortical layers. CSD is derived from the three signals (LFPs from channels a and c are subtracted from the 2× amplitude of channel b activity). The derived CSD signal is filtered (10 to 130 Hz), and signals exceeding the predetermined amplitude threshold are detected (spike detection). The thresholded signals are used to trigger TES, applied to the skull either in a bipolar configuration (left versus right hemispheres) or frontal midline versus parietal areas (as shown). (B) Example of LFP signal during a spontaneously occurring SW episode, CSD, and its filtered version in the absence of TES stimulation. (C) Similar SW episode in the presence of TES stimulation. The gating (red) “dead time” pulse (80 ms) was used to prevent prolonged spurious triggering of the stimulator during the wave components of SW episode.

Fig. 2. TES modulates cortical neuronal firing and spike component of SW. **(A)** Raster plot and histogram of multiple-unit firing during interictal 1-Hz TES (sinus). **(B)** Because both TES and SW strongly modulated unit firing, SW-related discharge was calculated in 10 bins (100-ms windows), using the spike component as reference in each bin. TES-induced cyclic modulation of multiple-unit firing during SW is shown. **(C)** TES modulation of the LFP spike of SW [different rat from (A) and (B)] and mean and SD of SW in each bin. **(D)** Polar plots of the magnitude of the LFP spike component in the absence (left, random phase) and presence of TES (right). (A) to (D) are single-session examples. **(E)** Group data showing the effectiveness of TES stimulation on unit-firing modulation during SW patterns. Modulation index (MI) of 1 corresponds to sessions in which TES completely silenced MUA in at least one bin. **(F)** Distribution of MI of the LFP spike amplitude compared with randomly shuffling the relationship between LFP spike amplitude and TES phase in all sessions and rats ($n = 9$ rats).

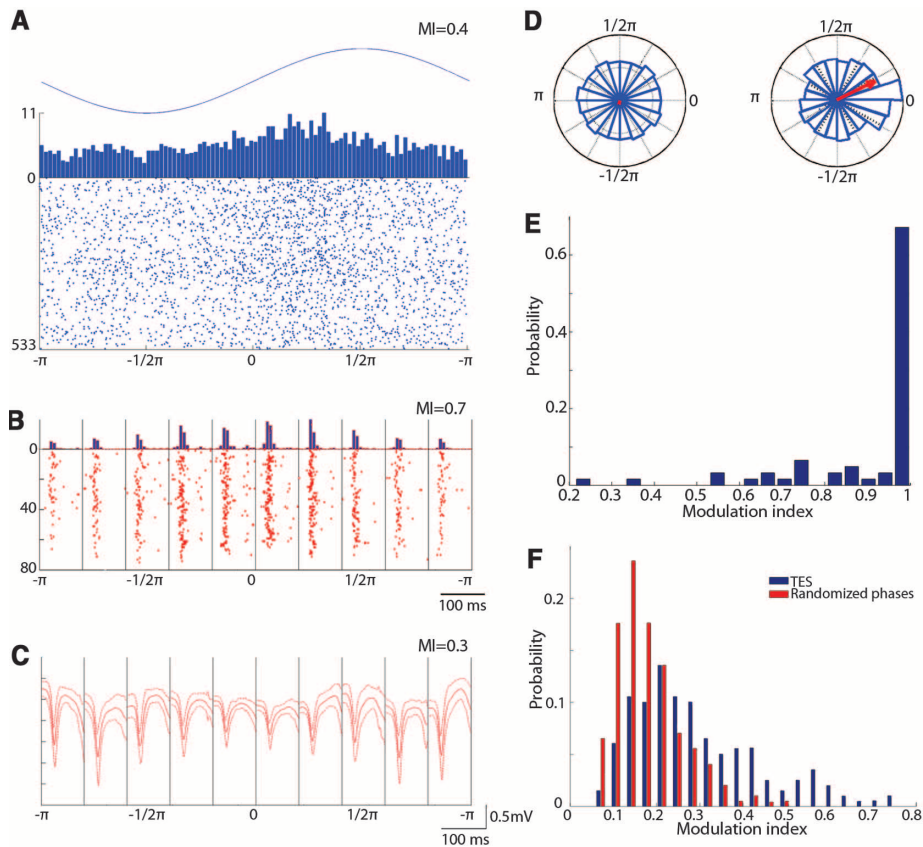
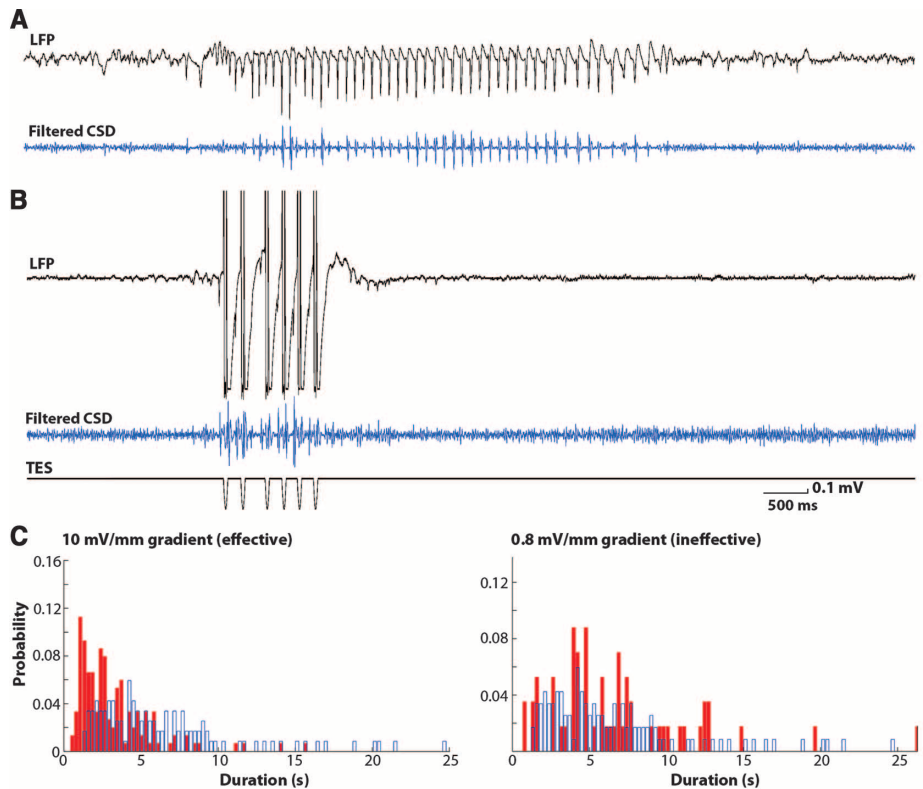


Fig. 3. Closed-loop feedback TES stimulation aborts SW episodes. **(A and B)** SW episodes (LFP and filtered CSD traces) without (top) and with (bottom) feedback TES. **(C)** Distribution of the duration of SW episodes in the absence (control, blue) and presence of feedback TES. Shown are data from two example sessions with effective (10 mV/mm voltage gradient intensity) and ineffective, subthreshold (0.8 mV/mm) TES stimulation.

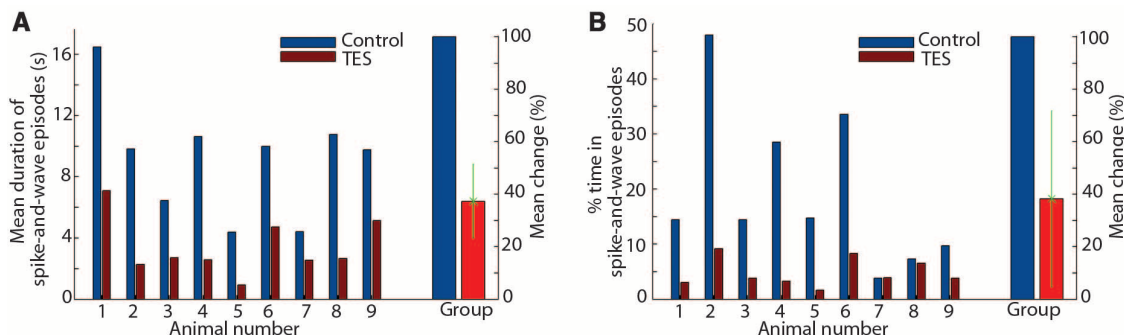


temporal electrodes, placed directly on the skull, or between these bitemporal electrodes, against a frontal midline electrode (Fig. 1B and fig. S1

(18). TES-induced artifacts were reduced by using tripolar electrodes and deriving current source density (CSD) of the recorded traces online (Fig. 1,

C and D). TES sinusoid trains at 1 Hz induced significant rate modulation of multiple unit firing patterns both in the absence and presence of

Fig. 4. Closed-loop feedback TES stimulation aborts SW episodes. **(A)** Mean duration of SW episodes in each of the nine rats tested and the group mean (and SD). **(B)** Percent time spent in SW episodes in a given recording session. Shown are results from individual rats and group. Mean percent change refers to group data.



spike-and-wave (SW) episodes ($P < 0.01$; 61 of 103 cortical sites; $n = 3$ rats; Rayleigh test) (Fig. 2). In addition to affecting the firing rates of neurons, TES at 1 Hz also strongly modulated the spike amplitude of SW patterns (Fig. 2, C and D, and fig. S2) but had no effect on the duration of SW episodes [time (t) = 8.62 s, 21,902 s of TES epochs; $t = 9.14$ s, 21,775 s of control epochs; $n = 9$ rats; $P > 0.05$; t test for dependent samples]. Additional control experiments demonstrated that TES, at the intensities used, neither induced arousal effects when applied during sleep nor affected overt behavior during waking, as demonstrated by the lack of TES-induced head movements (figs. S3 and S4).

We next sought to examine how brain activity-triggered stimulation affects SW patterns. Because SW patterns are strongly periodic events, involving reverberatory activity of the thalamocortical loop (14), we applied Gaussian waveforms of 50-ms TES after the detected spike components. SW-triggered TES shortened the duration of SW episodes in an intensity-dependent manner (Fig. 3). Group analysis of closed-loop TES stimulation showed that the mean duration of the SW episodes was significantly shorter in nine of the nine rats ($P < 0.01$; two sample t test). In addition, the percent time spent in SW episodes in a given session was significantly reduced in seven of the nine rats ($P < 0.01$). Overall, feedback TES stimulation lead to a $>60\%$ decrease of both the duration of SW episodes and the fraction of session time spent in SW across animals (Fig. 4). The percent decrease of time spent in SW episodes in a given session was largely a consequence of the decreased duration of SW episodes because there was a significant correlation between the mean duration and the fraction of time in SW episodes [correlation coefficient (r) = 0.60; $P < 0.001$; Pearson's correlation test] (fig. S5). This relationship thus shows that TES did not simply fragment or delay SW episodes. The number of SW episodes per unit time was not significantly different between control and TES sessions ($P > 0.05$; two sample t test), indicating that TES-induced reduction of SW episodes did not lead to a rebound or long-term compensatory increase of their probability of occurrence.

These findings show that brain pattern-triggered feedback TES of cortical neurons can

interfere with thalamocortical reverberation during SW episodes and effectively reduce their duration. SW patterns—the hallmark of generalized petit mal epilepsy—arise from complex interactions between thalamic and neocortical neurons (14, 22–24). During the wave component of the SW cycle, both neocortical and thalamic neurons are largely silent (22–25). We hypothesize that cortical excitatory feedback during this silent period, brought about with TES, quenched the ongoing rhythm by recruiting subsets of thalamic cells, which in turn became refractory during the duty phase of the native SW cycle, as shown through tandem optogenetic activation of the thalamus and neocortex in mice (fig. S6). Brain activity-timed feedback of TES appears critical because 1-Hz sinusoid trains did not affect the duration of SW episodes.

Successful clinical application of closed-loop TES has two fundamental requirements (9, 11, 26, 27). The first is the recording and identifying of causal pathophysiological network patterns. In generalized SW and focal cortical epilepsies, subdural or epidural electrodes, or even electrodes inserted into the skull, may be sufficient. In complex partial seizures, which make up the largest fraction of drug-resistant epilepsies, deep-electrode recordings are required for accurate detection of abnormal patterns (11, 13). The second requirement is closed-loop feedback stimulation of the target circuits, whose activation can interfere with the emerging pathological pattern. Intra-skull plate electrodes may ideally diffuse the applied currents to affect sufficiently large groups of neurons (28). Alternatively, multiple and appropriately placed plates may be used to achieve more focal concentration of current. In contrast to transcranial magnetic stimulation, which requires large and heavy coils, TES electrodes implanted in the skull and powered by ultralight electrical circuits are a cosmetically acceptable solution for long-term clinical applications. Noninvasive, closed-loop TES stimulation may also prove useful for improving mental and mood states.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S6
References (29–36)

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Shear-Activated Nanotherapeutics for Drug Targeting to Obstructed Blood Vessels

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Obstruction of critical blood vessels due to thrombosis or embolism is a leading cause of death worldwide. Here, we describe a biomimetic strategy that uses high shear stress caused by vascular narrowing as a targeting mechanism—in the same way platelets do—to deliver drugs to obstructed blood vessels. Microscale aggregates of nanoparticles were fabricated to break up into nanoscale components when exposed to abnormally high fluid shear stress. When coated with tissue plasminogen activator and administered intravenously in mice, these shear-activated nanotherapeutics induce rapid clot dissolution in a mesenteric injury model, restore normal flow dynamics, and increase survival in an otherwise fatal mouse pulmonary embolism model. This biophysical strategy for drug targeting, which lowers required doses and minimizes side effects while maximizing drug efficacy, offers a potential new approach for treatment of life-threatening diseases that result from acute vascular occlusion.

Disruption of normal blood flow to the heart, lung, and brain is the leading cause of death and long-term adult disability in the Western world (1). Current approaches to acute therapy for ischemic stroke, coronary infarction, and pulmonary embolism require infusion of thrombolytic drugs, which need to be administered systemically or through a catheter placed within the obstructed vessel, usually in an acute care hospital setting (2–4). To be effective, patients must receive therapy within a few hours after onset of symptoms, and the doses of clot-lysing drugs that can be administered are limited by the potential risk of bleeding because the active drug is free to distribute throughout the body. To overcome these limitations, we designed a thrombolytic delivery system that targets drugs selectively to sites of flow obstruction and concentrates the active drug in these regions.

Stenotic and thrombosed blood vessels exhibit physical characteristics that distinguish them from normal vasculature in that fluid shear stress can increase locally by one to two orders of magnitude, from below ~ 70 dyne/cm² in normal vessels to >1000 dyne/cm² in highly constricted

arteries (5–8). Normal circulating platelets are locally activated by high shear stress in these regions and rapidly adhere to the adjacent surface lining of the narrowed vessels (9–11), which is a major contributing factor in the development of vulnerable atherosclerotic plaques. Inspired by this natural physical mechanism of platelet targeting, we developed a therapeutic strategy that uses local high shear stress as a generic mechanism to target treatment to regions of blood vessels that are constricted by clots, stenosis, or developmental abnormalities.

Our shear-activated nanotherapeutics (SA-NTs) are similar in size to natural platelets (1 to 5 μ m in diameter); however, they are fabricated as aggregates of multiple smaller nanoparticles (NPs). The microscale aggregates remain intact when flowing in blood under physiological flow conditions but break up into individual nanoscale components when exposed to high local shear stress. Because of their smaller size as compared with that of the microscale aggregates, shear-dispersed NPs experience lower drag forces and, hence, adhere more efficiently to the surface of the adjacent blood vessel wall than do the larger microaggregates (fig. S1). The efficiency of this local adhesion can be further enhanced by coating the NPs with molecules that bind to endothelial cells or relevant targets, such as fibrin clots. In this manner, high concentrations of therapeutic agents can be concentrated locally at sites of vascular occlusion or embolism by immobilizing relevant drugs or enzymes on the NPs.

The SA-NTs were produced by spray-drying concentrated solutions of biocompatible, biodegradable, poly(lactic-co-glycolic acid) (PLGA 50:50, MW 17 kD) to form micrometer-sized (3.8 ± 1.6 μ m) aggregates composed of small (180 ± 70 nm) NPs (Fig. 1A). Microaggregates of PLGA NPs are stable in aqueous solutions because of their hydrophobicity (12, 13). But

when exposed to mechanical forces that overcome the attractive forces holding the NPs together, such as hemodynamic shear stresses, the aggregates break apart (Fig. 1B), much like a wet ball of sand disperses into individual grains when rubbed in one's hands.

To determine the shear-sensitivity of this NP deployment mechanism, we used a rheometer to apply controlled shear stresses in vitro to SA-NTs fabricated from NPs labeled with a fluorescent tag. We detected an 8- to 12-fold increase in the concentration of released NPs when the level of shear reached 100 dyne/cm² or higher (Fig. 1C). This range of fluid shear stress is relevant in many vascular diseases. For example, computational fluid dynamics (CFD) modeling of flow within normal and stenotic human left coronary arteries based on ultrasound imaging (supplementary materials, materials and methods) revealed that the level of shear that induces NP release in vitro is similar to that generated by a 60% lumen obstruction (Fig. 1D), whereas normal coronary vessels experience a 20% lower level of shear stress (~ 10 to 30 dyne/cm²) that does not cause disruption of the SA-NTs.

To determine whether these SA-NTs can target agents selectively to stenotic regions under relevant hemodynamic flow conditions, we carried out studies in a three-dimensional (3D) microfluidic model of vascular narrowing fabricated from poly-dimethylsiloxane (PDMS) that was designed to mimic regions of living blood vessels with 90% lumen obstruction (Fig. 2, A and B). Based on CFD modeling, such a constriction generates an ~ 100 -fold increase in shear at the stenotic site (Fig. 2C). Perfusion of SA-NTs [100 μ g/ml in phosphate-buffered saline (PBS)] through these microfluidic devices resulted in a 16-fold increase in the release of free NPs, as measured in the solution flowing downstream of the obstruction compared with fluid flowing through unobstructed microfluidic channels of similar dimensions (Fig. 2D). Moreover, fluorescence microscopic imaging confirmed that released NPs accumulated in endothelial cells cultured on the inner surface of the artificial microfluidic vessel just distal to the narrowed region, whereas minimal uptake occurred in cells lining the channel before the constriction (Fig. 2E).

To evaluate their functional potential, we fabricated SA-NTs containing fluorescent NPs coated with the U.S. Food and Drug Administration–approved thrombolytic drug, tissue plasminogen activator (tPA), using biotin-streptavidin chemistry ($\sim 5 \times 10^5$ tPA molecules/microaggregate) and tested their ability to dissolve blood clots. To examine the general utility of this shear-targeted nanotherapeutic approach (Fig. 3A) for removal of natural clots formed endogenously in vivo, we studied the effect of bolus injection of thrombolytic SA-NTs in an established mouse arterial thrombus model, in which clot formation is triggered by injuring the vessel wall by direct

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exposure to ferric chloride (14–16). Real-time, intravital, fluorescence microscopic studies confirmed that this treatment resulted in formation of large blood clots within minutes in injured mesenteric arteries ($\sim 100\ \mu\text{m}$ diameter, normal wall shear stress $\sim 30\ \text{dyne/cm}^2$) that occluded the diameter by more than 80% (Fig. 3, B and C) and caused the local shear stress to increase by more than 15-fold ($\sim 450\ \text{dyne/cm}^2$) in these regions, as determined by using an optical Doppler velocity meter (17). Fluorescently labeled

tPA-carrying SA-NTs that were injected intravenously 8 min after chemical injury preferentially accumulated in the regions of clot formation, resulting in clear microscopic visualization of these lesions (Fig. 3B). In addition, the locally deployed tPA-coated NPs induced progressive surface erosion of the thrombi, with complete clearance of occlusions occurring within 5 min after SA-NT injection (Fig. 3, B and C, and movie S1). Continuous monitoring of unobstructed vessels for up to 15 min in the mesenteric bed re-

vealed that intact microscale NP aggregates were present throughout the course of the study, indicating that circulation of the SA-NTs through the normal vasculature did not induce microaggregate disruption.

Shear-induced release of tPA-coated NPs from the SA-NTs reopened the obstructed mesenteric arteries and significantly delayed the time to vessel occlusion ($29 \pm 7\ \text{min}$ with tPA-coated SA-NTs versus $12 \pm 3\ \text{min}$ with PBS), when vessel patency was monitored by using intravenous injection of

Fig. 1. Microscale SA-NTs disperse into NPs only when exposed to pathological shear stresses. **(A)** Scanning electron micrographs of the microscale (~ 2 to $5\ \mu\text{m}$) SA-NTs (left) and the PLGA NPs ($\sim 180\ \text{nm}$) used to produce them (right). Scale bar, $2\ \mu\text{m}$. **(B)** Fluorescence micrographs demonstrating intact SA-NTs (top) and NPs dispersed after their exposure to $1000\ \text{dyne/cm}^2$ for 10 min by using a rheometer (bottom). Scale bar, $10\ \mu\text{m}$. **(C)** Quantification of release of fluorescent NPs from the SA-NTs as a function of shear revealed that exposure to pathological levels of shear ($\geq 100\ \text{dyne/cm}^2$ for 1 min) caused large increase in the breakup of the microscale aggregates into NPs compared with physiological levels of shear (1 or $10\ \text{dyne/cm}^2$) ($*P < 0.005$). **(D)** CFD simulations comparing fluidic shear stress in a normal coronary artery (left) and a stenotic vessel with a 60% lumen obstruction (right). Left inset shows the corresponding angiogram of the stenotic left coronary artery in a 63-year-old male patient.

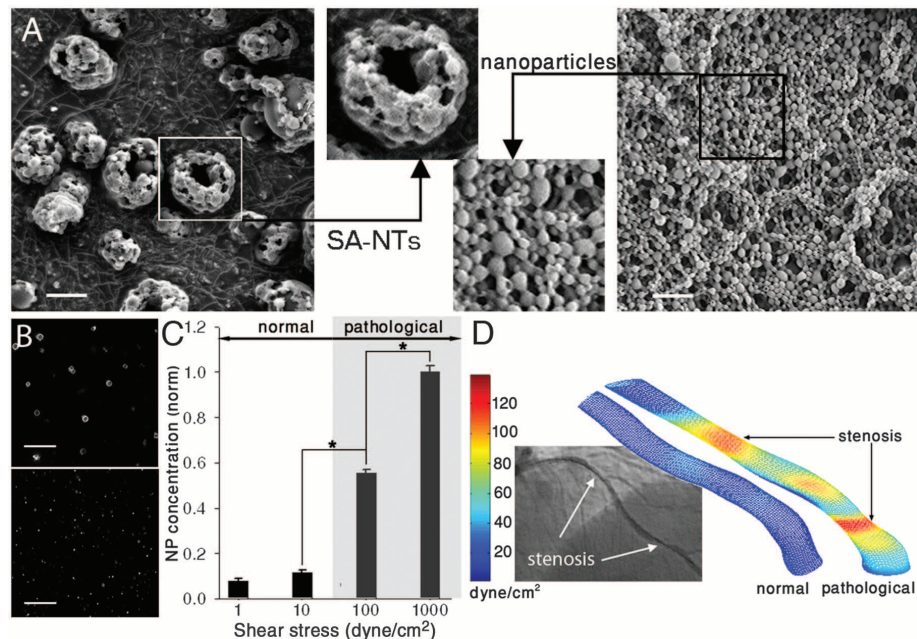
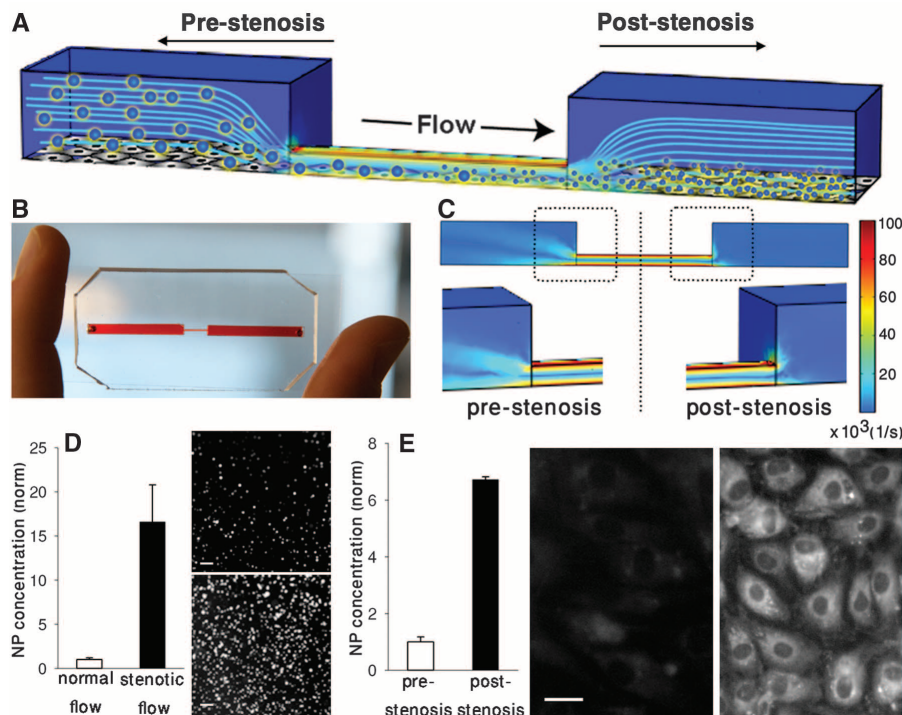


Fig. 2. Shear-induced dissociation of SA-NTs and NP targeting under hemodynamic conditions in microfluidic devices. **(A)** A microfluidic vascular stenosis model showing how SA-NTs (large spheres) should remain intact in the prestenotic region but then break up into NPs (small spheres) when they flow through a constriction (90% lumen occlusion) and can accumulate in endothelial cells lining the bottom of the channel. **(B)** A photograph of the microdevice that mimics vascular stenosis fabricated in PDMS. **(C)** CFD simulations of the microfluidic device shown in (B) demonstrating that a physiological inlet shear rate of $1000\ \text{s}^{-1}$ ($10\ \text{dyne/cm}^2$) upstream from the constriction increases to a pathological level of $\sim 100,000\ \text{s}^{-1}$ ($1000\ \text{dyne/cm}^2$) in the region displaying 90% lumen occlusion. **(D)** Graph shows a >10 -fold increase in release of fluorescent NPs from SA-NTs when they are perfused through the channel shown in (B) compared with flow through an unconstricted channel ($*P < 0.005$). Fluorescent micrographs compare the NPs collected in the outflow from the control channel (top) versus the constricted channel (bottom). Scale bar, $2\ \mu\text{m}$. **(E)** Graph demonstrates that many more fluorescent NPs accumulate in endothelial cells lining the downstream area (poststenosis) of the constriction relative to an upstream area ($P < 0.005$). Fluorescence microscopic images show cells from regions before (left) and after (right) the constriction. Scale bar, $20\ \mu\text{m}$.



fluorescently labeled platelets (~2.5% of total platelets) (Fig. 3, C and D, and movie S2). In contrast, administration of the same dose of soluble tPA (free tPA), predissociated tPA-NPs, or heat-fused tPA-NP microaggregates (which do not dissociate in high shear) did not delay thrombosis in this model (Fig. 3D). Careful analysis of these results also revealed that even when a vessel is almost fully occluded, the microscale tPA-coated SA-NTs that bind to the surface of the clot can actively degrade and “recanalize” the clot. Once this happens, flow and shear stress rapidly increase once again, and this feeds back to activate other tPA-carrying SA-NTs, resulting in full clot removal (Fig. 3C and movie S2). Taken together, these results provide proof-of-principle that the SA-NT technology can be used to target clot-lysing agents to vascular occlusions, in addition to providing a way to image these lesions in real time in situ.

To explore the potential value of the SA-NTs for treatment of life-threatening embolic occlusions, we first tested their ability to dissolve experimentally induced fibrin clots in vitro. When preformed fibrin clots [250 ± 150 - μ m diameter produced by a water-in-oil emulsion technique (18)] were injected into microfluidic channels that contained constricted regions (80 μ m high, 500 μ m wide), the fibrin emboli lodged in the devices and partially obstructed flow in the channels (Fig. 4A). When SA-NTs (100 μ g/ml) carrying tPA (50 ng/ml) were infused at physiological flow rates through the clot-occluded microfluidic channels, the shear-dispersed fluorescent tPA-coated NPs accumulated at the surface of the artificial emboli, progressively dissolving the clots and reducing their size by one half within an hour of treatment (Fig. 4A and movie S3). In contrast, treatment with soluble tPA at the same concentration and flow conditions had negligible effects (<5% reduction in clot size) (Fig. 4B).

Next, we tested the ability of this shear-activated tPA delivery system to reverse the effects of acute pulmonary embolism in an ex vivo whole-mouse-lung ventilation-perfusion model. A solution containing the preformed fibrin clots similar to those tested in the microfluidic channel were infused (0.1 ml/min for ~5 min; 1×10^3 clots/ml) through the pulmonary artery of the perfused lung. Occlusion of pulmonary blood vessels by multiple microemboli (Fig. 4C) caused the pulmonary artery pressure to increase by approximately threefold compared with its normal value (30 versus 8 mm Hg) (Fig. 4E). We then perfused tPA-coated SA-NTs (100- μ g/ml microscale aggregates containing NPs coated with 50 ng/ml tPA) through the pulmonary artery at a physiological flow rate (0.5 ml/min). Fluorescence microscopic analysis of tissue sections again confirmed that the tPA-NPs localized selectively at regions of vascular occlusion, producing a >25-fold increase in accumulation of NPs at these sites, (Figs. 4, C and D). Progressive lysis of the emboli by the tPA-NPs resulted in normalization of pulmonary artery pressure levels within

1 hour in this ex vivo model (Fig. 4E). In contrast, perfusion of soluble tPA at the same concentration as that delivered on the injected tPA-coated NPs (50 ng/ml), or even at a 10-times-higher dose (500 ng/ml), failed to produce any meaningful response (Fig. 4F). In fact, similar clot-lysing effects and hemodynamic changes were only observed when we administered a 100-times-higher concentration of soluble tPA under identical flow conditions (Fig. 4F); this dose in mice (~2 mg/kg) is comparable with the therapeutic dose commonly used in humans (~1 mg/kg).

We then studied pulmonary embolism in living mice by infusing smaller preformed fluorescent fibrin clots (<70 μ m; ~1000 clots) into the jugular vein of anesthetized mice, which accumulate in peripheral blood vessels in the lungs, as previously described (19). SA-NTs coated with tPA were then infused either immediately after injection of emboli or 30 min after they

formed. Quantitation of the total area of fluorescent emboli visualized in the lungs by use of computerized image analysis confirmed that administration of the tPA-coated SA-NTs resulted in reduction of both total clot area and clot number by >60% when administered immediately after injection of emboli and by >30% when infused one half hour after embolism (fig. S2).

To further examine the potential clinical relevance of our approach for treatment of life-threatening acute massive embolism, we infused a solution containing larger fibrin clots (150 ± 80 - μ m diameter; ~100/injection), which accumulate in the main pulmonary arteries (19) much as they do in humans with pulmonary embolism, and the mice were then immediately infused with tPA-coated SA-NTs or with carrier fluid for 45 min. All control animals died within 1 hour after infusion of the clots (0% survival, $n = 7$ mice) (Fig. 4G), whereas >80% of the treated mice sur-

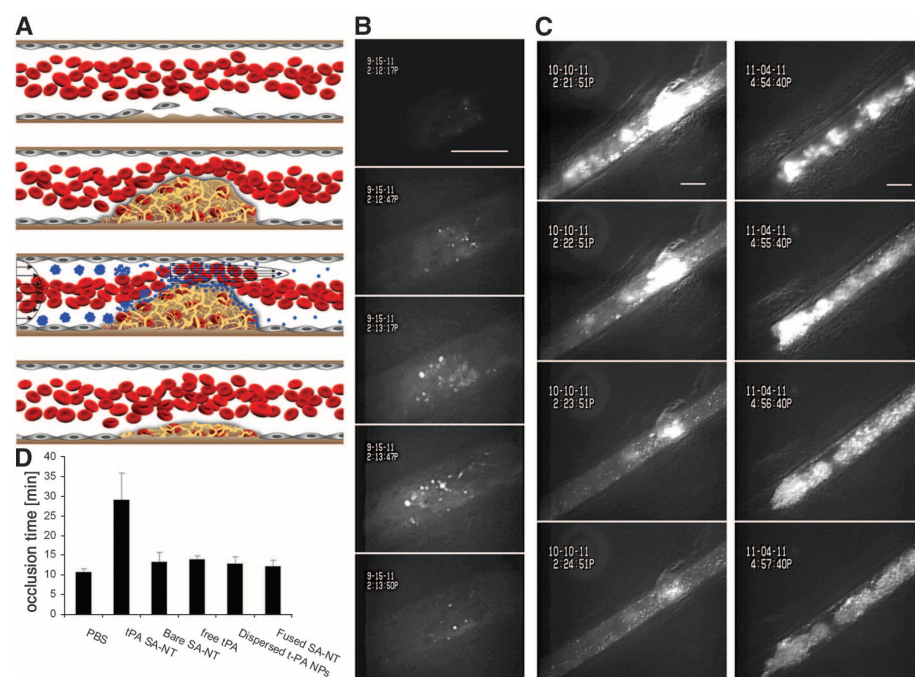


Fig. 3. Shear-targeting of a thrombolytic drug in a mouse arterial thrombosis model using SA-NTs. **(A)** Schematic of the experimental strategy. Ferric chloride injury initiates formation of a thrombus (top) that grows to partially obstruct blood flow (top middle). Intravenously injected SA-NTs dissociate into NPs at the thrombus site because of the rise in local shear stress (bottom middle). Accumulation of tPA-coated NPs and binding to the clot at the occlusion site progressively dissolve the obstruction (bottom). **(B)** Sequential intravital fluorescence microscopic images of a thrombus in a partially occluded mesenteric artery recorded over a 5-min period beginning after bolus injection of fluorescent tPA-coated SA-NTs (1 mg NPs; 50 ng tPA) 8 min after injury initiation. Scale bar, 100 μ m. The NPs accumulate at the clot, first visualizing its location and then demonstrating clearance of the clot within 5 min after injection at the bottom (movie S1). **(C)** Sequence of intravital fluorescence microscopic images recorded over a 5-min period showing fluorescently labeled platelets accumulated within a forming thrombus that partially occludes a mesenteric artery 8 min after injury. Thrombosis is then treated with injection of either tPA-carrying SA-NTs (50 ng tPA) (left) or PBS (right). Scale bar, 100 μ m. The clot on the left is greatly reduced in size within 5 min after SA-NTs injection (movie S2), whereas the control vessel on the right fully occludes over the same time period. **(D)** Graph showing that a bolus injection of SA-NTs carrying 50 ng tPA (tPA-SA-NT) significantly delayed the time to full vascular occlusion in FeCl₃-injured vessels ($***P < 0.0005$), whereas administration of the same concentration of soluble tPA (free tPA), uncoated SA-NTs (bare SA-NT), tPA-coated NPs that were artificially dissociated from SA-NTs before injection (dispersed tPA-NPs), and heat-fused NP microaggregates with tPA coating that do not dissociate (fused SA-NT) did not produce any significant delay in thrombosis.

vived (6 out of 7), and none of these SA-NTs-treated animals displayed any visible symptoms of respiratory distress.

The major potential advantage of the SA-NTs is their ability to enhance the safety of thrombolytic therapies by substantially reducing the drug dose required to be effective, as demonstrated by the ability of SA-NTs to clear pulmonary emboli when coated with a tPA dose $\sim 1/100$ th of that required for induction of similar clot-lysing effects by free tPA. SA-NTs also could help to minimize unwanted bleeding and neurotoxicity because they are cleared rapidly from the circulation (80% clearance in 5 min) (fig. S3), and because of their larger size, they should not diffuse as easily into injured tissues as free tPA does. Additionally, we

have not observed any abnormal bleeding in mice during the extensive surgical manipulations that were involved in our pulmonary embolism and mesenteric artery occlusion models. Nevertheless, before advancing these SA-NTs toward clinical studies in the future, finer control over the size of the microscale aggregates and their pharmacokinetics will be required to ensure that they safely pass through all microvessels and are sustained in the circulation at effective levels (20). Alternative methods to link tPA to NPs [such as direct conjugation by amine-carboxylate coupling or coupling based on biocompatible heterobifunctional polyethylene glycol linkers (21, 22)] also will need to be explored so as to avoid immune responses associated with streptavidin/biotin con-

jugation and to increase conjugation efficiency as well as optimize tPA activity.

A previously described thrombo-prophylaxis strategy based on coupling plasminogen activators to carrier erythrocytes has shown promising results in preventing thrombosis in various animal models (23–25). The SA-NTs described here can potentially be used to prevent formation of thrombi that partially occlude vascular flow, such as occurs, for example, when a stable atherosclerotic plaque is transformed into a life-threatening vulnerable plaque. However, in contrast to the erythrocyte delivery approach, which is limited to prevention of nascent clot formation, the shear-activated drug targeting strategy also offers the ability to treat and dissolve preexisting fibrin clots, such as those found in patients with stroke and myocardial infarction as well as atherosclerosis. In this proof-of-principle study, we focused on SA-NTs coupled to a thrombolytic agent, tPA, which has high affinity for fibrin, and thus, they were efficiently delivered to fibrin clots (fig. S1). In addition, these tPA-coated SA-NTs were loaded with a fluorescent dye that also concentrated at vascular occlusion sites. In the future, it should be possible to design and fabricate SA-NTs containing various drugs or imaging agents for localized treatment and real-time visualization in a variety of pathologies associated with vascular obstruction.

These findings illustrate how nanoengineering approaches inspired by pathophysiological mechanisms can be used to develop safer and more effective therapeutic strategies. In contrast to drug targeting mechanisms that focus on expression of distinct molecular species that can vary between tissues or patients, shear stress increases as a function of narrowing of the lumen diameter in all patients, regardless of the cause or location of obstruction, thus offering a robust and broadly applicable targeting strategy. With further refinement, the shear-activated drug targeting nanotechnology described here might, for example, allow the immediate administration of clot-busting drugs to patients suspected to have life-threatening clots in the brain, lung, or other vital organs by emergency technicians or other caregivers, even before the patient has reached a hospital setting.

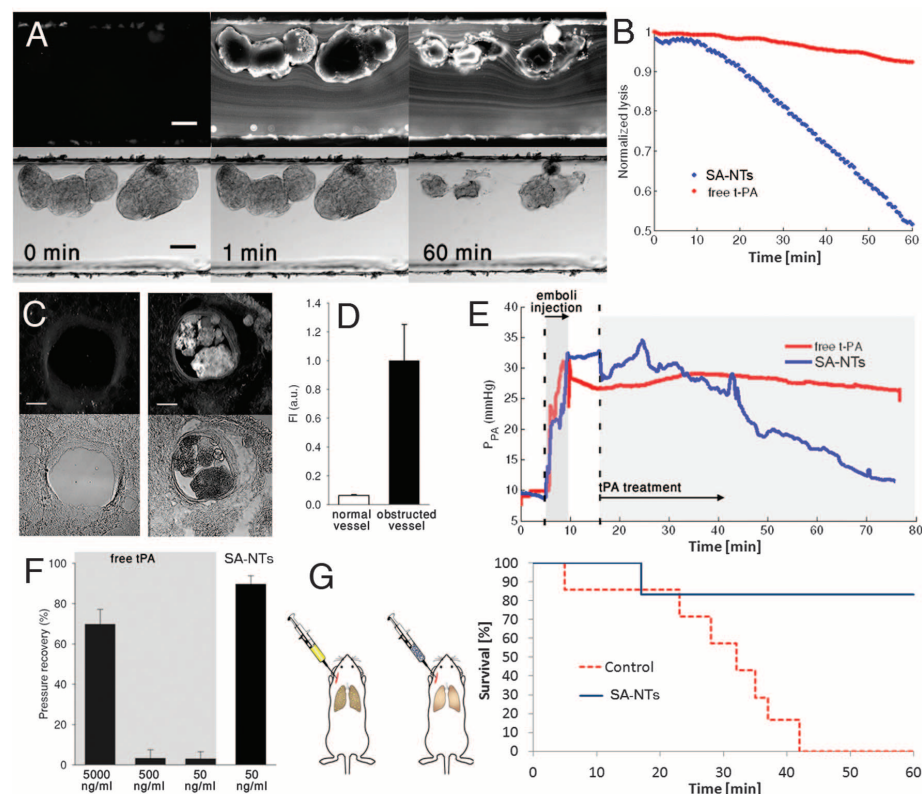


Fig. 4. Shear-targeting of a thrombolytic drug to vascular emboli in vitro and therapeutic delivery in a mouse pulmonary embolism model. (A) Time-lapse fluorescence (top) and bright field (bottom) views of artificial microemboli (~ 250 μm) in a microfluidic channel before (0 min) and 1 or 60 min after injection of SA-NTs coated with tPA (50 ng/ml), showing progressive lysis of the clots over time (movie S3). Scale bar, 100 μm . (B) Graph showing enhanced emboli lysis kinetics induced by tPA-coated SA-NTs (50 ng/ml, blue line) compared with soluble tPA (red line). (C) Fluorescence (top) and phase contrast (bottom) views of histological sections of normal (left) versus obstructed (right) pulmonary arteries showing local accumulation of fluorescent NPs within the obstructing emboli in a mouse ex vivo lung ventilation-perfusion model. Scale bar, 100 μm . (D) Graph showing almost a 20-fold increase ($P < 0.005$) in accumulation of fluorescent NPs in regions of obstructed versus nonobstructed vessels, as detected with microfluorimetry. (E) Real-time measurements of pulmonary artery pressure in the ex vivo pulmonary embolism model showing that the tPA-coated SA-NTs (blue line) reversed pulmonary artery hypertension within approximately 1 hour, whereas the same concentration (50 ng/ml) of free tPA was ineffective (red line). (F) Graph showing that tPA carrying SA-NTs normalize pulmonary artery pressure within 1 hour, whereas the same concentration of free tPA (50 ng/ml) or a 10-times-higher dose (500 ng/ml) did not reduce pulmonary artery pressure ($*P < 0.005$); only a 100-fold-higher dose (5000 ng/ml) produced similar effects. (G) Survival curve showing that almost all (86%) of the mice injected with the tPA-coated SA-NTs survived, whereas all control mice died within 45 min after injection of fibrin clots that caused acute emboli formation.

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M.K.) have filed two patents related to this work: (i) Shear-Activated Nanotherapeutics for Drug Targeting (patent application pending) and (ii) Shear Controlled Release for Stenotic Lesions and Thrombolytic Therapies (patent application pending WO 2012/074588).

Supplementary Materials

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Materials and Methods
Figs. S1 to S3
References (26–32)
Movies S1 to S3

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Drosophila Dosage Compensation Involves Enhanced Pol II Recruitment to Male X-Linked Promoters

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Through hyperacetylation of histone H4 lysine 16 (H4K16), the male-specific lethal (MSL) complex in *Drosophila* approximately doubles transcription from the single male X chromosome in order to match X-linked expression in females and expression from diploid autosomes. By obtaining accurate measurements of RNA polymerase II (Pol II) occupancies and short promoter-proximal RNA production, we detected a consistent, genome-scale increase in Pol II activity at the promoters of male X-linked genes. Moreover, we found that enhanced Pol II recruitment to male X-linked promoters is largely dependent on the MSL complex. These observations provide insights into how global modulation of chromatin structure by histone acetylation contributes to the precise control of Pol II function.

In *Drosophila* males, the histone acetyltransferase MOF (males absent on the first) within the MSL (male-specific lethal) complex mediates global hyperacetylation of X-linked chromatin at histone H4 Lys¹⁶ (H4K16) (1, 2). The mark is thought to mediate a doubling of X-linked transcription by promoting the formation of an accessible chromatin structure (3–6). However, the mechanism of this activation has remained elusive. It has been proposed that dosage compensation occurs by enhanced transcription elongation through male X-linked genes (7, 8), although direct support for this claim is limited.

The study of dosage compensation has been complicated by the relatively small (factor of 2) increase in transcription rate and by the widespread use of aneuploid male cell lines as the model

system. A shortcoming has been the lack of direct comparison of RNA polymerase II (Pol II) occupancies between male and female flies. Without reference measurements in females, sex-specific and gene-specific effects cannot be distinguished and unambiguous conclusions about the dosage compensation mechanism are impossible.

Chromatin immunoprecipitation sequencing (ChIP-seq) experiments for the Rpb3 subunit of Pol II were performed in male and female third-instar larva salivary glands (table S1 and figs. S1 and S2). We integrated these measurements with ChIP-seq data for MOF, histone H4, and acetylated H4K16 (H4K16ac) from the same tissues (9). We clustered 1360 autosomal and 242 X-linked genes with significant Pol II occupancies along the full transcribed regions according to their H4K16ac status in males (Fig. 1A and fig. S3). As expected, binding by the acetyltransferase MOF correlated with the occurrence of H4K16ac. Moreover, although the histone mark extends across the entire lengths of genes on the male X chromosome, it is restricted to gene promoters on the male autosomes and on all female chromosomes (10).

A comparison of Pol II occupancies between the gene clusters in male and female samples reveals the difference in transcriptional output achieved through dosage compensation (Fig. 1B

and fig. S4). There is a clear enrichment of Pol II levels among hyperacetylated genes on the male X chromosome; this enrichment is maintained throughout the entire length of genes, including promoter regions [defined as transcription start site (TSS) –300 base pairs (bp) to TSS +500 bp], transcribed regions [TSS +500 bp to polyadenylation site (PolyA) –500 bp], and 3' ends (PolyA –500 bp to PolyA +300 bp). These results support the strong correlation between chromosome-wide H4K16ac and increased transcriptional activity on the male X chromosome.

To identify the precise step of the Pol II transcription cycle that is modulated by the dosage compensation mechanism, we generated averaged metaprofiles of Pol II occupancies for the male autosomal and X-chromosomal genes (Fig. 2A). For all chromosomes, there are prominent peaks of Pol II association at promoters, with maxima about 50 bp downstream of the TSS that reflect widespread promoter-proximal stalling of Pol II (11, 12). We observed a factor of 2.2 enrichment in Pol II occupancies at the promoters of male X-linked genes relative to autosomal ones [Fig. 2, A (lower panel) and E, and fig. S5C]. Pol II levels were also higher by a factor of 2.3 throughout the transcribed regions of male X-linked genes [Fig. 2, A (lower panel) and E]. Similar results were obtained using the male-derived embryonic S2 cell line (table S1 and figs. S5, A and D).

To account for the potential influences of gene-specific regulation, we compared these profiles against female controls by generating Rpb3 ChIP-seq profiles in female third-instar larva salivary glands. In contrast to males, the female pattern of Pol II occupancies was similar for both X-linked and autosomal genes (Fig. 2, B and E, and fig. S5C). The X-to-autosomal enrichment of Pol II occupancy at promoters was on average higher in males than in females by a factor of 1.9 (Fig. 2E). In gene-by-gene comparisons of promoter occupancies, male X-linked genes showed a significant shift toward higher rankings relative to females (Fig. 2C, $P < 2.2 \times 10^{-16}$). This increase in Pol II levels was maintained beyond the promoters, through the transcribed regions (factor of 2.2 increase) and 3' ends (factor of 2.3 increase) of male X-linked genes. The results are robust: Analysis using a larger set of

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Fig. 1. Pol II enrichment on the hyperacetylated male X chromosome. **(A)** Heat map of MOF binding and H4K16ac across genes in male (left) and female (right) samples. Genes are divided into three groups according to male wild-type H4K16ac levels: fully acetylated X-linked genes (top, 242 genes), promoter-acetylated autosomal genes (middle, 1157 genes), and nonacetylated autosomal genes (bottom, 203 genes). The TSS is indicated by a dashed line. Only expressed genes with significant Pol II signal in their transcribed region are shown. **(B)** Heat map showing male versus female differences ($\log_2$) in Pol II occupancies in the promoter (TSS –300 bp to TSS +500 bp) (left), transcribed regions (TSS +500 bp to PolyA –500 bp, scaled to the same length) (center), and 3' ends (PolyA –500 bp to PolyA +300 bp) (right).

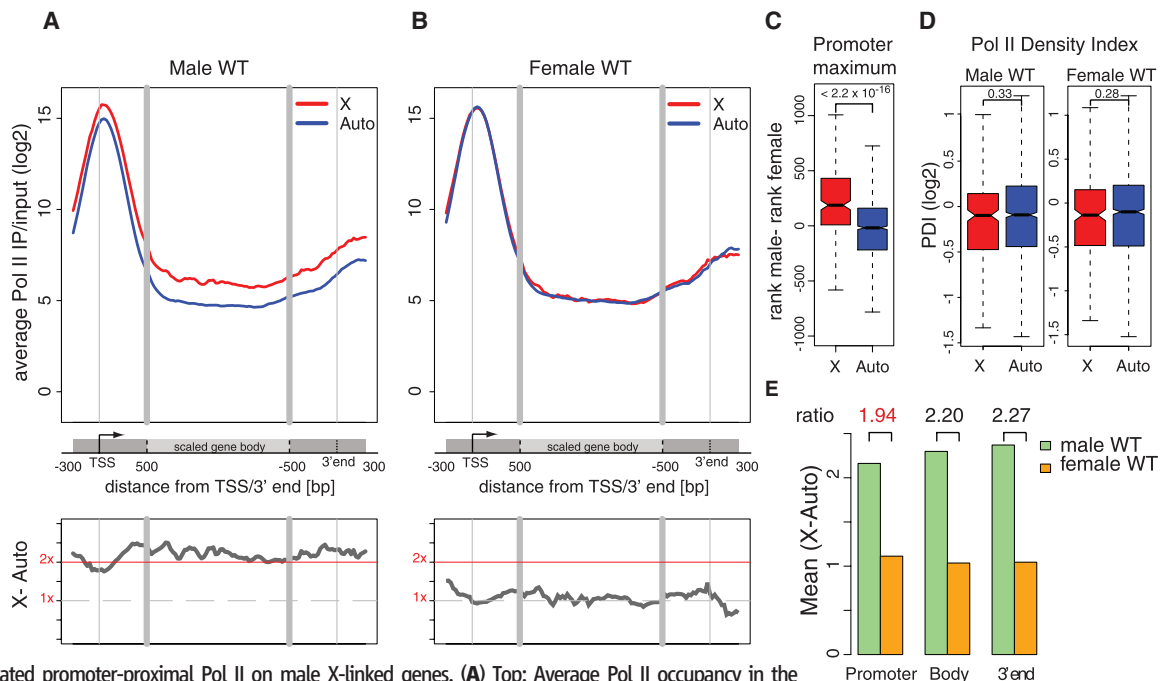
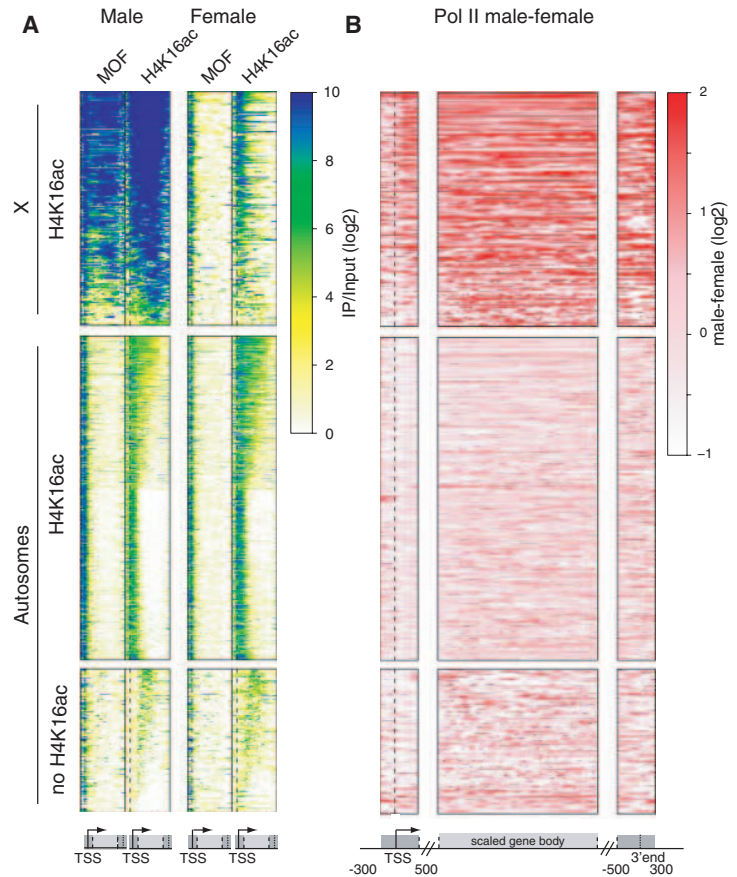


Fig. 2. Elevated promoter-proximal Pol II on male X-linked genes. **(A)** Top: Average Pol II occupancy in the promoter, middle, and 3' end of X-linked (red) and autosomal (blue) genes in wild-type (WT) males. Bottom: Relative difference between the two profiles. Thin gray lines represent the TSS and PolyA sites, respectively. Expressed genes with significant Pol II signals in their transcribed regions are included (254 X-linked and 1414 autosomal genes for the promoter and gene body; 96 X-linked and 406 autosomal genes for the 3' end, because 3' ends with neighboring genes were excluded). **(B)** Same analysis for wild-type females. **(C)** Gene-by-gene comparison of Pol II occupancies. Box plots show male versus female differences in rank orders of maximal Pol II occupancies at promoters. **(D)** Box plots of Pol II density indices (PDIs) for genes in male and female samples. **(E)** Bar plots comparing the mean X versus autosome Pol II occupancy ratios in wild-type males and females at the promoter, middle, and 3' end of genes. Indicated at top are the male versus female ratios of X-to-autosome Pol II occupancies. *P* values (Wilcoxon rank-sum test) are indicated in the box plots.

genes defined as expressed by microarrays and displaying significant Pol II signals at their promoters (639 X-linked and 3178 autosomal)—irrespective of significant ChIP-seq signal in gene bodies—revealed a factor of 2.0 increase in promoter-proximal Pol II occupancies among male X-linked genes (Fig. 3A). Analogous results were also obtained when genes were stratified by their expression levels (fig. S6 and table S2). Further, X-chromosomal promoters that were also acetylated in females (214 genes) showed a factor of 2.1 increase in Pol II occupancies in males (fig. S7, A, B, and D); the enhanced Pol II recruitment to these promoters correlated with a male-specific increase in H4K16ac upstream of the TSS (fig. S7, E and F). Absolute measurements of Pol II occupancy by quantitative polymerase chain reaction (qPCR) at individual gene

promoters validated the genome-wide observations (Fig. 3C). Any regulatory effects at the level of Pol II recruitment should also influence initiated but transcriptionally stalled polymerases (11–13). In agreement with this, we observed a factor of 2.0 enrichment in Pol II occupancies at the promoters of stalled X-linked genes in males (Fig. 3C). This suggests that the dosage compensation mechanism operates in the absence of productive elongation. More than one-third of *Drosophila* genes give rise to short (35- to 60-nucleotide) 5' RNA transcripts that reside within—and indicate the presence of—fully initiated but temporarily stalled Pol II (13). Using a panel of 15 stalled genes, we measured an average factor of 2.1 increase in the production of short transcripts from male X-linked

promoters relative to females (Fig. 3D). To test the specificity of these assays, we checked that the qPCR reactions generated unique products of expected sizes and confirmed the absence of confounding mRNA fragments (Fig. 3D and table S3). A further 15 actively transcribed genes displayed an average factor of 1.7 increase in short RNA production from male X-linked promoters (fig. S8). These results provide strong evidence for enhanced transcription initiation in the hyperacetylated environment of the male X chromosome. A previous study generated global run-on sequencing (GRO-seq) data in male S2 cells to report elevated transcription elongation among X-linked genes (8). To assess whether there are any changes in Pol II occupancies along the transcribed length of genes, we calculated a Pol II

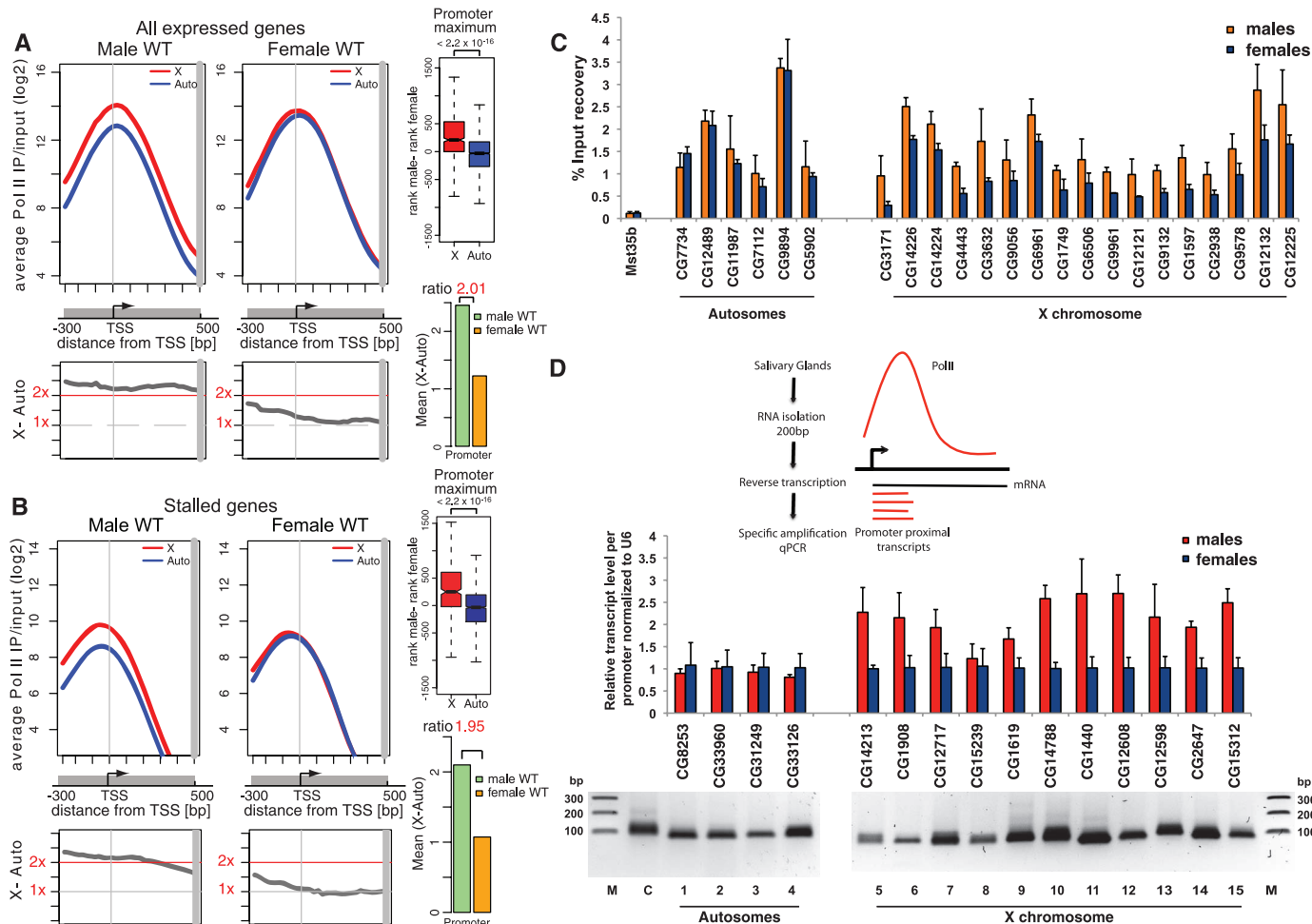


Fig. 3. Enhanced promoter activity is a general feature of X-linked genes. **(A)** Average Pol II occupancy around the TSS (TSS –300 bp, TSS +500 bp) using all expressed X-linked genes (red, 639 genes) and autosomal genes (blue, 3178 genes) in males and females. Box plots show male versus female differences in rank orders of maximal Pol II occupancies at promoters. Bar plots compare the mean X versus autosome Pol II occupancy ratios in males and females. **(B)** Same analysis for stalled genes, defined as unexpressed genes with significant Pol II occupancy at the promoter (395 X-linked and 1914 autosomal genes). **(C)** ChIP-qPCR validations in males and females. Pol II levels were measured as percent input recovery at 6 autosomal (left) and 17 X-linked gene promoters (right). The testis-specific *Mst35b*

gene serves as a negative control. Male X-linked promoter occupancy is elevated on average by a factor of 1.8. Error bars represent SD of three independent replicates. **(D)** Top: Short promoter-proximal RNAs were isolated, reverse-transcribed, and specifically quantified by qPCR. Middle: Relative transcript levels were measured for 15 transcriptionally stalled X-linked and autosomal genes. Error bars represent SD of three independent replicates. Bottom: Quality control for the specificity of short RNA detection using DNA gels with single product amplification. M indicates the molecular weight size marker; C indicates control U6 RNA. Note that a 64-bp DNA linker is added during reverse transcription to allow detection with a universal reverse primer. *P* values (Wilcoxon rank-sum test) are indicated in the box plots.

density index (PDI) as the ratio between total Pol II occupancies in the last three quarters versus the first quarter of the gene body (see supplementary materials). In contrast to expectations, our ChIP-seq data revealed similar PDI values for all chromosomes in both salivary glands and S2 cells (Fig. 1B, Fig. 2D, and fig. S5B). Moreover, in agreement with a recent report (14), reanalysis of the GRO-seq data above shows that transcriptional activities along gene bodies are equivalent for all large chromosomal arms (Fig. 4A), except for chromosome 3R and the heterochromatic chromosome 4. The differences in PDIs between the X chromosome and autosomes that remain after excluding the unusual chromosome 4 are resistant to MSL2 depletion (Fig. 4B). However, in support of our observations, reanalysis of the GRO-seq results using the same gene set as the ChIP-seq analysis in S2 cells (846 X-linked and 3869 autosomal) confirms the significantly

enhanced activity of X-linked promoters (figs. S5E and S9A).

To test whether enhanced Pol II recruitment to X-linked promoters is MSL-dependent, we performed Rpb3 ChIP-seq after RNA interference (RNAi)-mediated down-regulation of MSL2 in male salivary glands (figs. S1C and S3C). Promoter-proximal Pol II occupancies on the X chromosome were reduced by a factor of 1.8 to near-autosomal levels (Fig. 4, C, D, and F, and fig. S5C), and Pol II levels in gene bodies were decreased by a factor of 1.9 (Fig. 4F). Analogous results were obtained for different gene sets (fig. S10). That the loss of compensation was incomplete probably reflects gene-specific transcriptional buffering and gene-specific feedback regulation in the absence of MSL-mediated dosage compensation (15). In agreement with previous observations, this buffering was more evident for weakly expressed genes (table S2) (15). MSL2 depletion

also caused a general decrease in Pol II densities along the transcribed region of genes (Fig. 4E); however, all chromosomes were similarly affected, suggesting a secondary effect unrelated to dosage compensation.

Taken together, our data suggest that dosage compensation mainly arises from a near-doubling of Pol II activity at the promoters of male X-linked genes, most likely reflecting enhanced transcription initiation on the male X chromosome. In addition, release of Pol II into gene bodies is also slightly enhanced, which suggests that the transition into elongation might be facilitated. An initiation-based model readily explains the recent observation that the promoter sequence and associated transcription factor-binding sites influence the susceptibility of a reporter to dosage compensation (16). It is also consistent with the global hyperacetylation of male X-linked promoters and intergenic regions, which elevates the general accessibility of regulatory

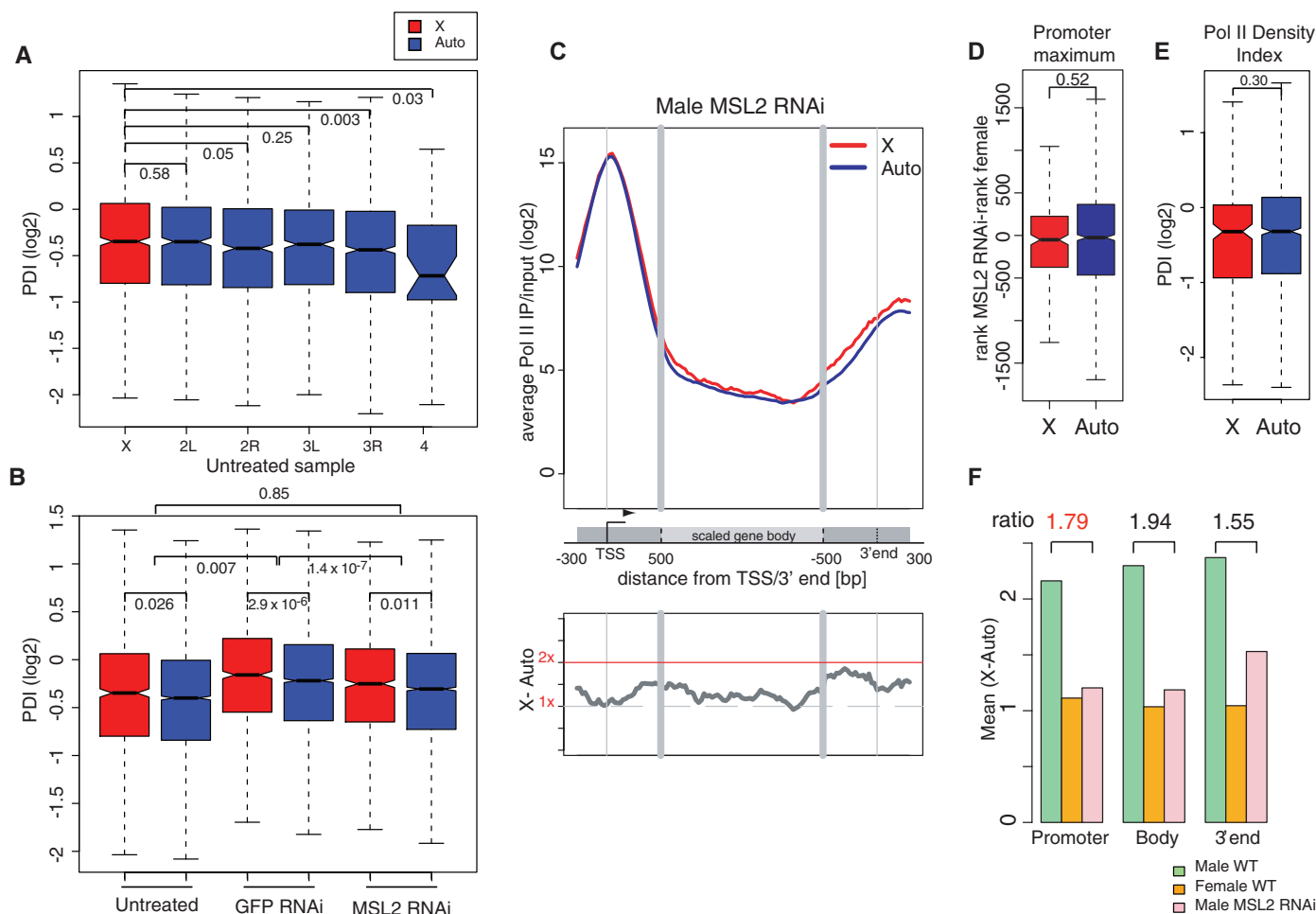


Fig. 4. Enhanced promoter activity is dependent on the MSL complex. (A) Box plots show the distribution of PDI values ($\log_2$) for each chromosome arm using publicly available GRO-seq data derived from S2 cells by Larschan *et al.* (8). (B) Box plots show the distribution of PDI values ($\log_2$) for X-linked genes (red) and autosomal genes (blue) in untreated, green fluorescent protein (GFP)-treated, and MSL2 RNAi-treated S2 cells using GRO-seq data by Larschan *et al.* (8). Genes on the heterochromatic chromosome 4 have been removed. (C) Top: Average Pol II occupancy in the promoter, middle, and 3' end of X-linked (red) and autosomal (blue) genes in MSL2 RNAi males detected by ChIP-seq against

Rpb3 (this study). Bottom: Relative difference between the two profiles. The same genes as in Fig. 2A were used. (D) Box plots show MSL2 RNAi male versus female differences in rank orders of maximal Pol II occupancies at promoters. (E) Box plots of PDI values for genes in MSL2 RNAi male and female samples. (F) Bar plots comparing the mean X versus autosome Pol II occupancy ratios in wild-type males and females and MSL2 RNAi males at the promoter, middle, and 3' end of genes. Indicated at top are the wild-type male versus MSL2 RNAi ratios of X-to-autosome Pol II occupancies. *P* values (Wilcoxon rank-sum test) are indicated in the box plots.

sequences to the transcriptional machinery (2, 5). At the same time, hyperacetylation within gene bodies is a prerequisite for MSL complex binding to the transcribed regions of X-linked genes (*1*). Restricting the MSL complex to these sites may prevent the physical obstruction of promoters in males and thereby help to balance the transcriptional output between sexes.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1221428/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 to S3
References (17–25)

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Fate-Restricted Neural Progenitors in the Mammalian Cerebral Cortex

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During development of the mammalian cerebral cortex, radial glial cells (RGCs) generate layer-specific subtypes of excitatory neurons in a defined temporal sequence, in which lower-layer neurons are formed before upper-layer neurons. It has been proposed that neuronal subtype fate is determined by birthdate through progressive restriction of the neurogenic potential of a common RGC progenitor. Here, we demonstrate that the murine cerebral cortex contains RGC sublineages with distinct fate potentials. Using *in vivo* genetic fate mapping and *in vitro* clonal analysis, we identified an RGC lineage that is intrinsically specified to generate only upper-layer neurons, independently of niche and birthdate. Because upper cortical layers were expanded during primate evolution, amplification of this RGC pool may have facilitated human brain evolution.

The mammalian cerebral cortex consists of six major layers that each contain specific subtypes of neurons characterized by distinct projection patterns and gene expression profiles (*1*). These layer-specific classes of cortical excitatory neurons are derived from radial glial cells (RGCs) in sequential order, with neurons destined for lower layers being generated first, followed by upper-layer neurons and, finally, cortical astrocytes (*1*). This relationship between birthdate and laminar fate of neurons in the cerebral cortex has been documented for more than 50 years, although it has remained unclear whether cell fate is determined directly by birthdate (2) or if the two are linked more indirectly rather than causally (3).

RGCs divide asymmetrically in the cortical ventricular zone to self-renew and generate neurons directly or, more commonly, indirectly via intermediate progenitor cells that divide

symmetrically in the ventricular and subventricular zones (4). The transcription factor *Cux2* is expressed specifically in neurons in upper layers II to IV in the mature cortex (Fig. 1A), but also in intermediate progenitors in the developing subventricular zone (5, 6), suggesting that upper-versus lower-layer fate might be determined before neuronal differentiation. We found that *Cux2* mRNA is also expressed in the ventricular zone in a salt-and-pepper manner (Fig. 1B and fig. S1, A to D), indicating that some RGCs may be committed to generate upper-layer neurons. To establish the identity of ventricular zone *Cux2*⁺ cells and to determine their lineage potential, we employed genetic fate mapping using a mouse strain expressing Cre recombinase from the *Cux2* locus (7). Crossing the *Cux2-Cre* driver line to reporter mouse lines led to recombination primarily in upper-layer neurons in the mature cortex, with 76% of recombined cells occupying layers II to IV (Fig. 1C and fig. S1, E to H). Only 17 and 7% of recombined cells were found in lower layers V and VI, respectively (Fig. 1C and fig. S1, E to H), and most of these were *Satb2*⁺ excitatory neurons (74%) (fig. S1, I and J), which form callosal projections similar to layer II and III neurons (8, 9). The remainder were *Gad65/67*⁺ interneurons (26%) (fig. S1, I and J) derived from the ganglionic eminences, in agree-

ment with previous observations (6). Furthermore, in the developing cortex we identified clonal columns of recombined cells in the ventricular zone (Fig. 1D), resembling the pattern of endogenous *Cux2* mRNA expression. The majority of recombined cells in the ventricular zone expressed the RGC markers *Pax6* (Fig. 1E) and *nestin* (Fig. 1F), but not the intermediate progenitor marker *Tbr2* (Fig. 1G). Recombined cells divided at the ventricular surface (Fig. 1, H and I), maintained apical and basal processes, and underwent interkinetic nuclear migration (Fig. 1I), all hallmarks of ventricular zone RGCs. These results suggest that a subset of RGCs are restricted in their lineage potential.

To further analyze the relationship between *Cux2*⁺ RGCs and their offspring, we introduced a Cre reporter plasmid into RGCs in *Cux2-Cre* embryos by *in utero* electroporation. In this FLEX (FLip-Excision) technology-based reporter plasmid (10), Cre recombination switches expression from tdTomato fluorescent protein to green fluorescent protein (GFP), thereby permitting differential fluorescent labeling of *Cux2*⁺ and *Cux2*[−] RGCs and their offspring (Fig. 2A). Electroporation of the reporter at embryonic day (E) 12.5 and analysis at E13.5 demonstrated that a subset of electroporated RGCs had recombined the reporter and turned on GFP (Fig. 2B), even though at this early time point the tdTomato signal remained because of protein perdurance. At postnatal day (P) 10, 83% of neurons with recombined reporters (GFP⁺) had settled in upper layers II to IV (Fig. 2, C and D), whereas only 7% of the neurons with nonrecombined reporters (tdTom⁺GFP[−]) were located in upper layers (Fig. 2, C and D). These results suggest that the *Cux2*⁺ progenitors constitute a lineage largely fated to become upper-layer neurons with a small contribution to lower layers, whereas the *Cux2*[−] lineage primarily generates lower-layer neurons.

To facilitate temporal fate mapping of the *Cux2*⁺ lineage, we generated a mouse line in which the tamoxifen-inducible *CreERT2* (11) gene is knocked into the endogenous *Cux2* locus (fig. S2, A to C). By crossing *Cux2-CreERT2* mice with a Cre-reporter strain and inducing recombination

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Fig. 1. Expression of *Cux2* in a subset of RGCs suggests multiple progenitor types. (A and B) In situ hybridization shows *Cux2* mRNA at P7 (A) and E14.5 (B). Arrows indicate expression in a subset of ventricular zone cells. (C and D) Cumulative fate mapping of the *Cux2* lineage in *Cux2-Cre;Ai9* mice at P10 (C) and E14.5 (D). Arrows indicate recombination in a subset of ventricular zone cells. (E and F) Recombination in *Pax6*⁺ (E) and *nestin*⁺ (F) RGCs. (G) Recombination in *Tbr2*⁺ intermediate progenitors (arrowheads) and *Tbr2*[−] RGCs (arrows) in the ventricular zone. (H) Recombined mitotic cells dividing at the ventricular surface. (I) Live imaging of a slice culture from a *Cux2-Cre;Ai9* embryo showing a recombined RGC (arrow) undergoing interkinetic nuclear migration and dividing at the ventricular surface (dotted line). Arrowheads, apical and basal radial processes. LV, lateral ventricle; SVZ, subventricular zone; VZ, ventricular zone. Scale bars, 100 μ m [(C) and (D)] and 10 μ m [(E) to (H)].

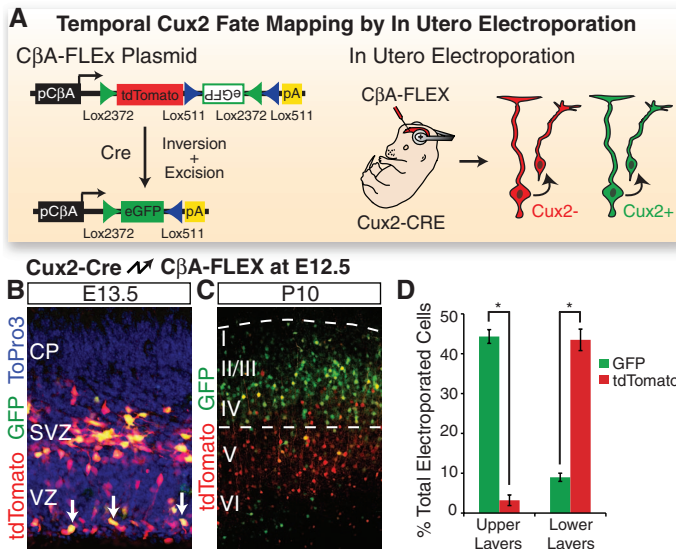
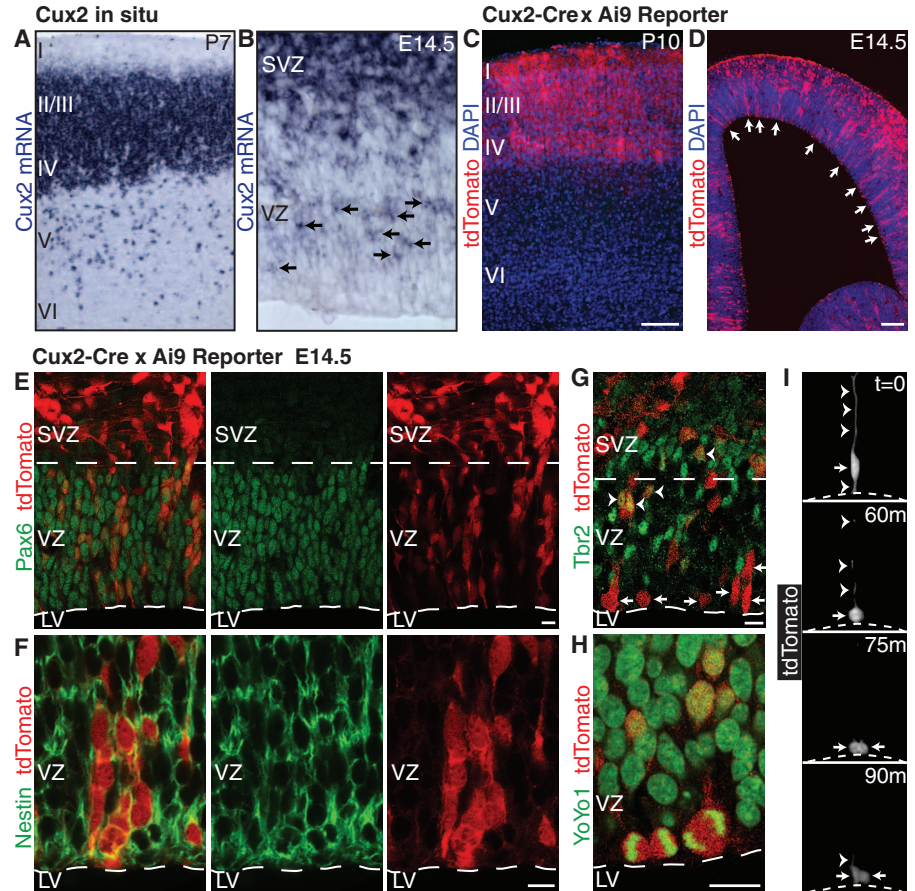
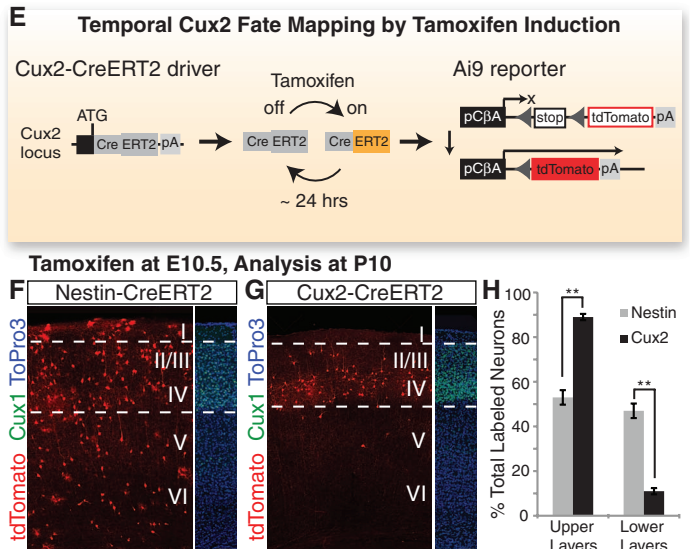


Fig. 2. RGCs of the *Cux2* lineage are fated to generate upper-layer neurons. (A) Temporal fate mapping by in utero electroporation. Upon in utero electroporation into *Cux2-Cre* embryos, CBA-FLEX drives differential expression of tdTomato and enhanced GFP (eGFP) in *Cux2*[−] and *Cux2*⁺ cells, respectively. (B) Electroporation of CBA-FLEX at E12.5, analysis at E13.5. Arrows identify cells in the ventricular zone that have recombined the plasmid. (C) Electroporation at E12.5, analysis at P10. (D) Percentage ($\pm$ SEM) of electroporated GFP⁺ and tdTomato⁺ neurons in upper versus lower layers at P10. * $P < 0.0005$. (E) Temporal fate mapping by tamoxifen induction. *Cux2*-



CreERT2 mice allow temporary activation of CreERT2 in the *Cux2*⁺ lineage within 6 to 24 hours after tamoxifen injection. CreERT2 activates the *Ai9* reporter to allow permanent tdTomato labeling during this window, but not before or after. (F) E10.5 induction, P10 analysis of a *Nestin-CreERT2* line, which drives recombination in *Cux2*⁺ and *Cux2*[−] RGCs. Dotted lines frame *Cux1* expression in upper layers. (G) E10.5 induction, P10 analysis of the *Cux2-CreERT2* line. (H) Percentage ($\pm$ SEM) of recombined neurons in upper versus lower layers for each driver. * $P < 1 \times 10^{-10}$. CP, cortical plate. Scale bars, 100 μ m.

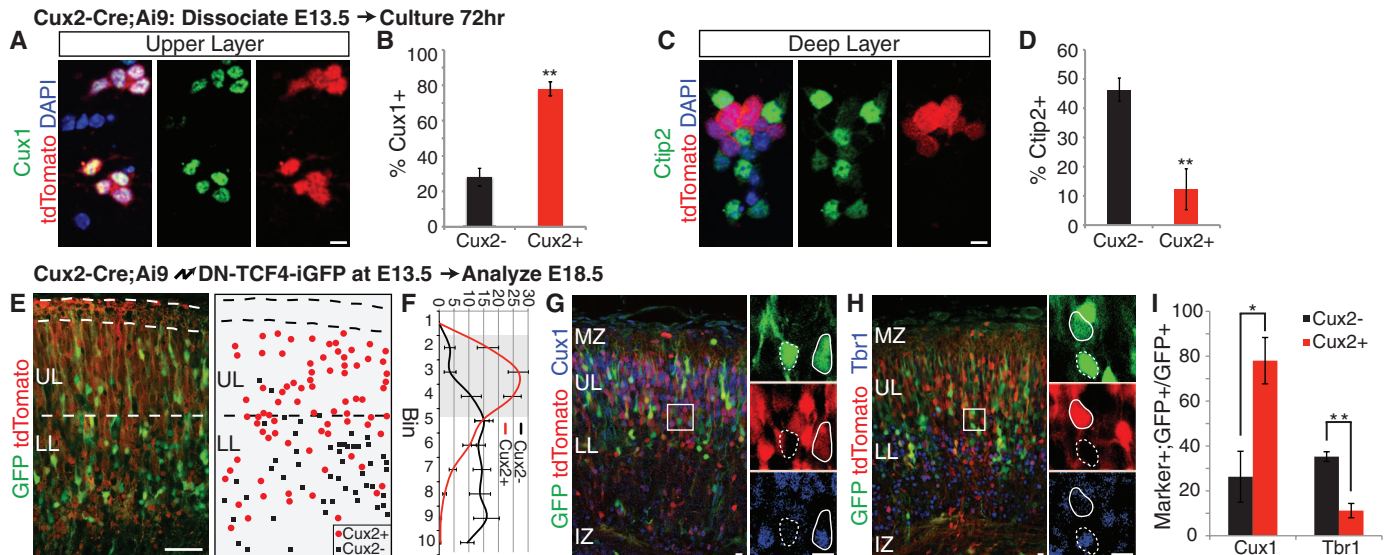
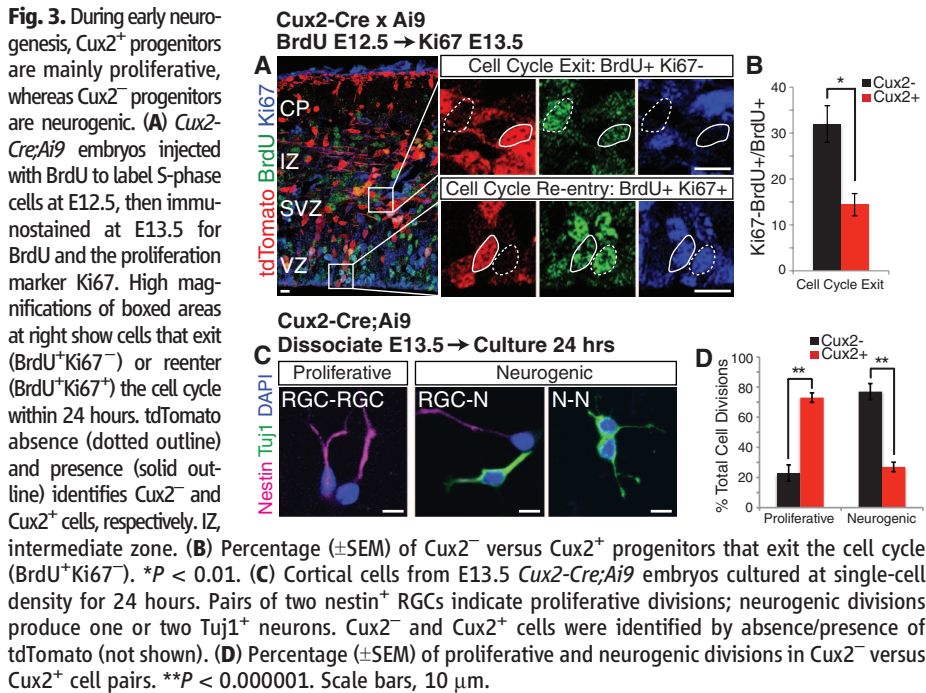
with a single injection of tamoxifen (Fig. 2E), we can label the $Cux2^+$ lineage within a narrow ~24-hour window (12, 13). Because most cortical cells are RGCs at E10.5, we injected tamoxifen at this time point to permanently label $Cux2^+$ RGCs between E10.5 and E11.5, but not before or afterward. We performed the same experiments with *Nestin-CreERT2* mice (14), which express tamoxifen-inducible Cre in all RGCs (fig. S2, D and E).

Whereas the *Nestin-CreERT2* line labeled neurons that contributed to all cortical layers (Fig. 2, F and H), as well as astrocytes and oligodendrocytes (fig. S2, F and G), the *Cux2-CreERT2* line labeled predominantly neurons in upper layers (Fig. 2, G and H). Thus, $Cux2^+$ RGCs are fated to generate upper-layer neurons even at the earliest stages of cortical neurogenesis. $Cux2^-$ RGCs are likely the proposed bipotent neuroglial progeni-

tors (15), or are perhaps further subdivided into lineage-restricted subtypes.

Lower-layer neurons are largely born before upper-layer neurons, although there is some degree of overlap in the birthdates of different neuronal subtypes (16). Because $Cux2^+$ upper-layer progenitors and $Cux2^-$ lower-layer progenitors coexist at the earliest stages of cortical development (fig. S3), we reasoned that during lower-layer neurogenesis, $Cux2^+$ RGCs might show a greater propensity to proliferate to expand the RGC pool, whereas $Cux2^-$ RGCs might preferentially generate neurons. In support of this idea, the percentage of $Cux2^+$ RGCs increased over time, whereas the percentage of $Cux2^-$ RGCs decreased (fig. S3). This suggests that the fraction of RGCs leaving the cell cycle at early stages of corticogenesis is lower for $Cux2^+$ RGCs than for $Cux2^-$ progenitors. To test this hypothesis, we analyzed cell cycle exit in *Cux2-Cre;Ai9* mice using a bromodeoxyuridine (BrdU) pulse to label S-phase cells at E12.5, followed by Ki67 staining at E13.5 to label cells that remained in the cell cycle (Fig. 3A). As expected, the fraction of $Cux2^+$ progenitors exiting the cell cycle was less than 50% that of $Cux2^-$ progenitors (Fig. 3B).

Because environmental cues such as notch-delta signaling control RGC self-renewal (17, 18), we hypothesized that notch signaling may be preferentially active in $Cux2^+$ RGCs. However, notch signaling was not differentially regulated in $Cux2^+$ versus $Cux2^-$ RGCs (fig. S4, A to D), and activation of the pathway affected the two populations similarly (fig. S4, E to G). Rather, the proliferative behaviors of the two RGC subtypes



appear to be at least partially intrinsic, because these differences were maintained outside their normal niche. When cells from E13.5 *Cux2-Cre;Ai9* embryos were cultured in vitro at clonal density (Fig. 3C), the majority of *Cux2*⁺ progenitors divided symmetrically to generate pairs of RGCs, whereas *Cux2*[−] progenitors preferentially underwent neurogenic divisions (Fig. 3D). We conclude that at early stages of corticogenesis, *Cux2*⁺ RGCs fated to generate upper-layer neurons preferentially proliferate to enlarge the precursor pool, whereas *Cux2*[−] RGCs already generate lower-layer neurons.

We next asked whether the generation of distinct neuronal subtypes is an intrinsic property of the two different progenitor types. Dissociated cortical cells from *Cux2-Cre;Ai9* embryos were cultured until they differentiated and were stained with layer-specific markers (Fig. 4, A and C). The in vivo situation was recapitulated in vitro, with ~80% of the *Cux2*⁺ progenitors generating neurons with upper-layer identity (Fig. 4B) and the majority of the *Cux2*[−] progenitors producing neurons with lower-layer identity (Fig. 4D). Thus, the neurogenic differences between the two progenitor populations are maintained outside their normal developmental niche, indicating an intrinsic aspect to their divergent fate specification. This result also suggests that birthdate may not be causative to cell fate. To test this hypothesis, we electroporated dominant-negative TCF4 into *Cux2*⁺ RGCs to force their premature cell cycle exit (19), so that they generated neurons at the expense of self-renewal (fig. S5). These prematurely generated *Cux2*⁺ neurons still preferentially occupied upper layers (Fig. 4, E and F) and expressed upper-layer, but not lower-layer, markers (Fig. 4, G to I), indicating that their fate was not altered by the change in birthdate. Thus, *Cux2*⁺ RGCs are intrinsically specified to generate upper-layer neurons, independent of niche or birthdate.

Our data show that a subset of RGCs is specified to generate upper-layer neurons regardless of birthdate, but these progenitors are intrinsically programmed to generate neurons predominantly later than their lower-layer counterparts. Thus, contrary to the prevailing model (2), our study indicates that molecular fate specification ensures proper birth order, rather than vice versa. Our data also suggest that the minor fraction of callosal projection neurons found in lower layers is derived from the same RGC pool as the major population of callosal neurons in upper layers, demonstrating a common lineage for these functionally similar neurons, irrespective of cortical layer position. Although this model applies to the broad RGC subclasses that generate intracortical versus subcortical/subcerebral projection neurons, it remains possible that the potential of *Cux2*⁺ and *Cux2*[−] progenitors is subsequently progressively restricted to further specify neuronal subtypes within the two lineages.

Upper cortical layers are expanded in primates and are required for high-level associative

connectivity. Defects in their function are implicated in the etiology of cognitive syndromes such as schizophrenia and autism. The subventricular zone of primates, and especially humans, is enlarged compared with other species and contains outer subventricular RGCs that are thought to generate increased numbers of upper-layer cortical neurons in the primate brain (20). Our findings suggest that an equally important evolutionary advance was the subdivision of labor among RGCs in the ventricular zone to generate lower- and upper-layer neurons.

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Bergmann Glial AMPA Receptors Are Required for Fine Motor Coordination

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The impact of glial neurotransmitter receptors in vivo is still elusive. In the cerebellum, Bergmann glial (BG) cells express α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs) composed exclusively of GluA1 and/or GluA4 subunits. With the use of conditional gene inactivation, we found that the majority of cerebellar GluA1/A4-type AMPARs are expressed in BG cells. In young mice, deletion of BG AMPARs resulted in retraction of glial appendages from Purkinje cell (PC) synapses, increased amplitude and duration of evoked PC currents, and a delayed formation of glutamatergic synapses. In adult mice, AMPAR inactivation also caused retraction of glial processes. The physiological and structural changes were accompanied by behavioral impairments in fine motor coordination. Thus, BG AMPARs are essential to optimize synaptic integration and cerebellar output function throughout life.

Astroglial cells sense synaptic activity through various neurotransmitter receptors and are considered to modulate neuronal processing (1–3). In the cerebellar cortex, ectopic release of glutamate from climbing and parallel fiber terminals activates Ca²⁺-permeable α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs) expressed on Bergmann glial (BG) appendages (4) that tightly enwrap Purkinje cell (PC) synapses

(5). Conversion of BG AMPARs into Ca²⁺-impermeable receptors by adenoviral-mediated delivery of the GluA2 gene (6) caused BG process retraction from PC synapses and altered PC excitatory postsynaptic currents (EPSCs), providing insight into the functional interaction between BG and glutamatergic synapses. However, whether BG AMPAR signaling influences cerebellar function in vivo has remained unknown.

To investigate the impact of BG AMPARs on cerebellar function, we generated inducible double knockouts (dKOs) to ablate GluA1 and GluA4 (*Gria1* and *Gria4*) AMPAR subunits selectively in astrocytes in a temporally controlled fashion. We crossed mice expressing the tamoxifen-sensitive Cre-recombinase CreERT2 under the astrocyte-specific, endogenous GLAST (Slc1a3)-promoter (7) with mice carrying loxP-flanked *Gria1* (8) and *Gria4* (9) alleles (Fig. 1A). Because BG cells express only the AMPAR subunits GluA1 and GluA4 (10, 11), inactivation

of both subunits will result in a complete loss of AMPAR function.

We injected mice ($GLAST^{CreERT2/+} \times Gria1^{fl/fl} \times Gria4^{fl/fl}$, termed dKO) with tamoxifen at postnatal day 14 (P14) for 5 days and studied BG AMPAR ablation 10 to 14 days after treatment (Fig. 1A). Control mice were homozygously floxed for both subunits, but these mice either lacked CreERT2 expression ($GLAST^{+/+}$) and were injected with tamoxifen (tam control) or expressed CreERT2 and received oil (oil control). We achieved an almost-complete loss of GluA1 and GluA4 expression in the molecular layer (ML) of dKO mice as assessed by confocal analysis (Fig. 1, B to I). We also determined a reduction of 85 and 50% of GluA1 and GluA4 α (12) mRNA expression, respectively, and ~70% of total GluA1/4L (12) protein expression in cerebellar homogenates (fig. S1, A and B).

The loss of functional BG AMPARs was confirmed by whole-cell recordings and puff application of AMPA with D-aspartate to activate both AMPARs and glutamate transporters (Fig. 1J). BG cells of tam and oil controls exhibited similar transporter [DL-threo- β -benzyloxyaspartate (TBOA)-sensitive] and AMPAR [6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione (NBQX)-sensitive] currents. However, in dKO mice, AMPAR currents were completely abolished (Fig. 1, J and K). Although in $GLAST^{CreERT2/+}$ cerebellar homogenates mRNA and protein levels were reduced, confocal analysis of GLAST immunolabeling revealed no overt differences (fig. S2), and BG cells of dKO and oil controls had comparable transporter currents to tam con-

trols, although they are heterozygous for the GLAST locus (Fig. 1K). Thus, functional membrane expression of glutamate transporters was unaltered and should not influence any phenotype observed in dKO mice.

The gross cerebellar organization was unaffected when BG AMPARs were lost in the third postnatal week of cerebellar development (fig. S3). However, because Ca^{2+} signaling of BG AMPARs is important for synaptic transmission of PC synapses (6), we asked whether selective loss of BG AMPAR signaling influenced synaptic input to PCs. We analyzed parallel fiber (PF)-evoked PC currents (PF-EPSCs) in dKO mice and found an increase in amplitude, half-width, and decay time (Fig. 2, A and B). As expected, increase of half-width and decay time was more pronounced in dKO mice after blockade of AMPAR desensitization (fig. S4). These data point to an impaired clearance of synaptically released glutamate resulting from BG process retraction at PC spines (6). Indeed, light microscopic and ultrastructural inspection revealed that BG processes were, to a large extent, retracted from PC spines and lacked their characteristic complex morphology (Fig. 2E and fig. S5). Together with previous findings (6), these data emphasize the importance of glutamate and Ca^{2+} -mediated modulation of the cytoskeleton in perisynaptic astroglial processes (13). Moreover, we found a decrease in miniature EPSC frequency (Fig. 2, C and D) in PCs of dKO mice. Ultrastructural analysis of PF-PC synapse density confirmed a reduction 12 days after treatment (Fig. 2E and fig. S6). Additionally, dKO mice

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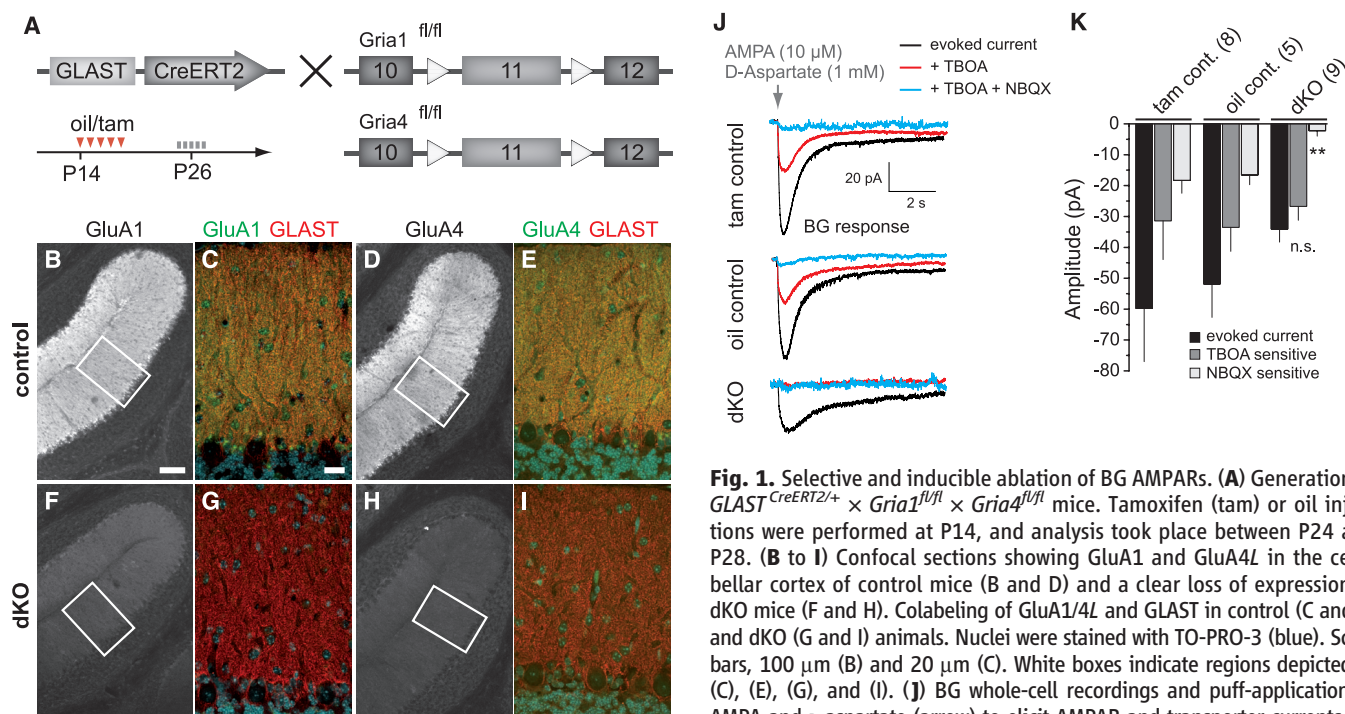


Fig. 1. Selective and inducible ablation of BG AMPARs. (A) Generation of $GLAST^{CreERT2/+} \times Gria1^{fl/fl} \times Gria4^{fl/fl}$ mice. Tamoxifen (tam) or oil injections were performed at P14, and analysis took place between P24 and P28. (B to I) Confocal sections showing GluA1 and GluA4L in the cerebellar cortex of control mice (B and D) and a clear loss of expression in dKO mice (F and H). Colabeling of GluA1/4L and GLAST in control (C and E) and dKO (G and I) animals. Nuclei were stained with TO-PRO-3 (blue). Scale bars, 100 μ m (B) and 20 μ m (C). White boxes indicate regions depicted in (C), (E), (G), and (I). (J) BG whole-cell recordings and puff-application of AMPA and D-aspartate (arrow) to elicit AMPAR and transporter currents. By applying TBOA and NBQX, transporter and AMPAR currents were dissected and quantified in (K). BG cells of dKO mice showed complete loss of AMPAR currents, whereas transporter currents remained unaltered compared with tam and oil controls. ** $P < 0.01$; ns, not significant.

and quantified in (K). BG cells of dKO mice showed complete loss of AMPAR currents, whereas transporter currents remained unaltered compared with tam and oil controls. ** $P < 0.01$; ns, not significant.

showed reduced levels of vesicular glutamate transporter (VGlut1), a marker for PF terminals (Fig. 2F) (14). Yet, there were no signs of inflammation, activated microglia, or apoptosis (fig. S7). At 30 (instead of 12) days postinjection, PF-PC synapse density in dKO mice reached control levels (fig. S6A), whereas BG process retraction was still very obvious (fig. S6B). We conclude that during cerebellar development, PF-PC synapse formation is at least partially regulated by BG AMPAR signaling. Our results provide additional in vivo evidence to previous findings that astrocytes are involved in synapse formation (15, 16). We could not, however, detect any differences in PF-PC synaptic plasticity such as paired pulse facilitation or long-term depression (figs. S8 and S9). We also did not observe an increase in climbing fiber terminals (fig. S10), but we cannot exclude a more subtle effect on elimination of multiple PC climbing fiber innervations (6).

At first glance, ablation of BG AMPARs during cerebellar development (at P14) did not cause any obvious ataxia. However, when dKO mice were challenged by running on a simple horizontal ladder, they revealed motor coordination deficits (fig. S11A). Unfortunately, the observed phenotype in young mice could have potential-

ly been biased by side effects of the tamoxifen treatment, because it influenced body-weight gain compared with oil-treated animals (fig. S11B). Hence, we thoroughly analyzed cerebellar motor behavior in adult injected animals, because body weight of adult mice was unaffected by tamoxifen treatment.

To obtain a more robust gene expression–behavior relation, we quantified the time courses of tamoxifen-induced gene excision, mRNA degradation, and protein loss and correlated these with the accompanied structural and behavioral alterations. Recombination kinetics differed between the floxed *Gria1* and *Gria4* alleles: Maximal gene recombination for *Gria1* was achieved 3 days after the first injection, whereas for *Gria4* it required more than 7 days (fig. S12C). Similarly, mRNA and protein loss was faster for GluA1 (Fig. 3A and fig. S12D). For both subunits, complete loss of BG AMPAR expression was achieved 3 weeks after tamoxifen treatment (Fig. 3A and fig. S13), demonstrating a clear difference in turnover kinetics of BG AMPAR subunits in young and adult mice (fig. S14).

We next evaluated whether AMPAR inactivation in adults caused BG process retraction from PC spines. Three weeks after tamoxifen treatment, we could not observe any overt pro-

cess retraction in dKO mice, although GluA1/A4 subunit expression was already completely lost (Fig. 3, B and D). However, 3 months after treatment, dKO mice displayed a significant reduction in BG appendage area and retraction from PC synapses (Fig. 3, C and D).

Does the loss of BG AMPAR signaling and subsequent retraction from PC synapses influence cerebellar function? We investigated the motor behavior of adult dKO mice at different periods after injections. We chose two cerebellum-related behavioral tasks, including locomotion conditioning on the Erasmus Ladder (Neurasmus BV, Rotterdam, Netherlands) (Fig. 4A) (12, 17) and Pavlovian eyeblink conditioning (18, 19), both of which allowed us to assess motor performance and motor learning in dKO mice during the same paradigm (Fig. 4 and figs. S15 and S16). First, we ruled out side effects of tamoxifen treatment on motor behavior by testing motor performance before and directly after injections (Fig. 4B). Performance was identical in both control groups as compared with inducible dKO mice (at that stage, there was no loss of AMPARs), indicating that the treatment itself did not affect motor performance. At 3 weeks postinjections, when loss of AMPARs was completed, we did not yet observe an overt difference in motor performance

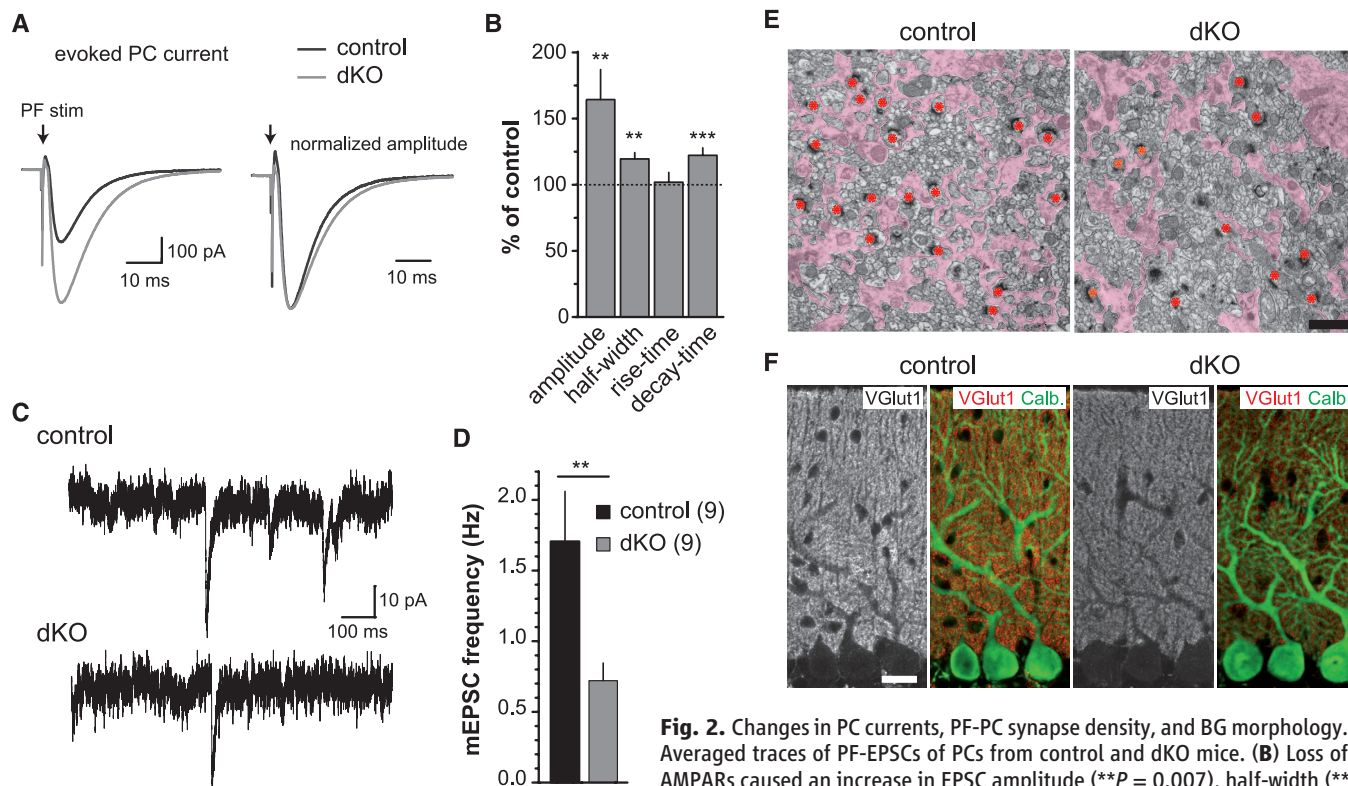


Fig. 2. Changes in PC currents, PF-PC synapse density, and BG morphology. (A) Averaged traces of PF-EPSCs of PCs from control and dKO mice. (B) Loss of BG AMPARs caused an increase in EPSC amplitude (** $P = 0.007$), half-width (** $P = 0.009$), and decay time (** $P < 0.001$); $n = 36$ to 40 cells per genotype. (C) Example traces of miniature EPSC (mEPSC) recordings from PCs (control, top; dKO, bottom) in 10 μ M gabazine and 500 nM tetrodotoxin to block inhibitory input and action potential–evoked responses. (D) PCs from dKO mice showed reduced mEPSC frequency by $57.7 \pm 7.3\%$ ($n = 9$; ** $P = 0.009$). (E) Ultrastructural analysis of PF-PC synapses in the upper third of the ML in control (left) and dKO (right) cells. BG processes are false-colored in pink, and synapses indicated with red asterisks. BG process complexity, synapse coverage, and synapse density were markedly reduced in dKO mice. Scale bar, 1 μ m. (F) Confocal sections showing VGlut1 (for PF synapses) and calbindin (for PCs) in control (left) and dKO (right). VGlut1 expression was decreased by $36.3 \pm 7.3\%$ ($n = 7$ versus 8; $P = 0.0003$) in the ML of dKO mice. Scale bar, 20 μ m.

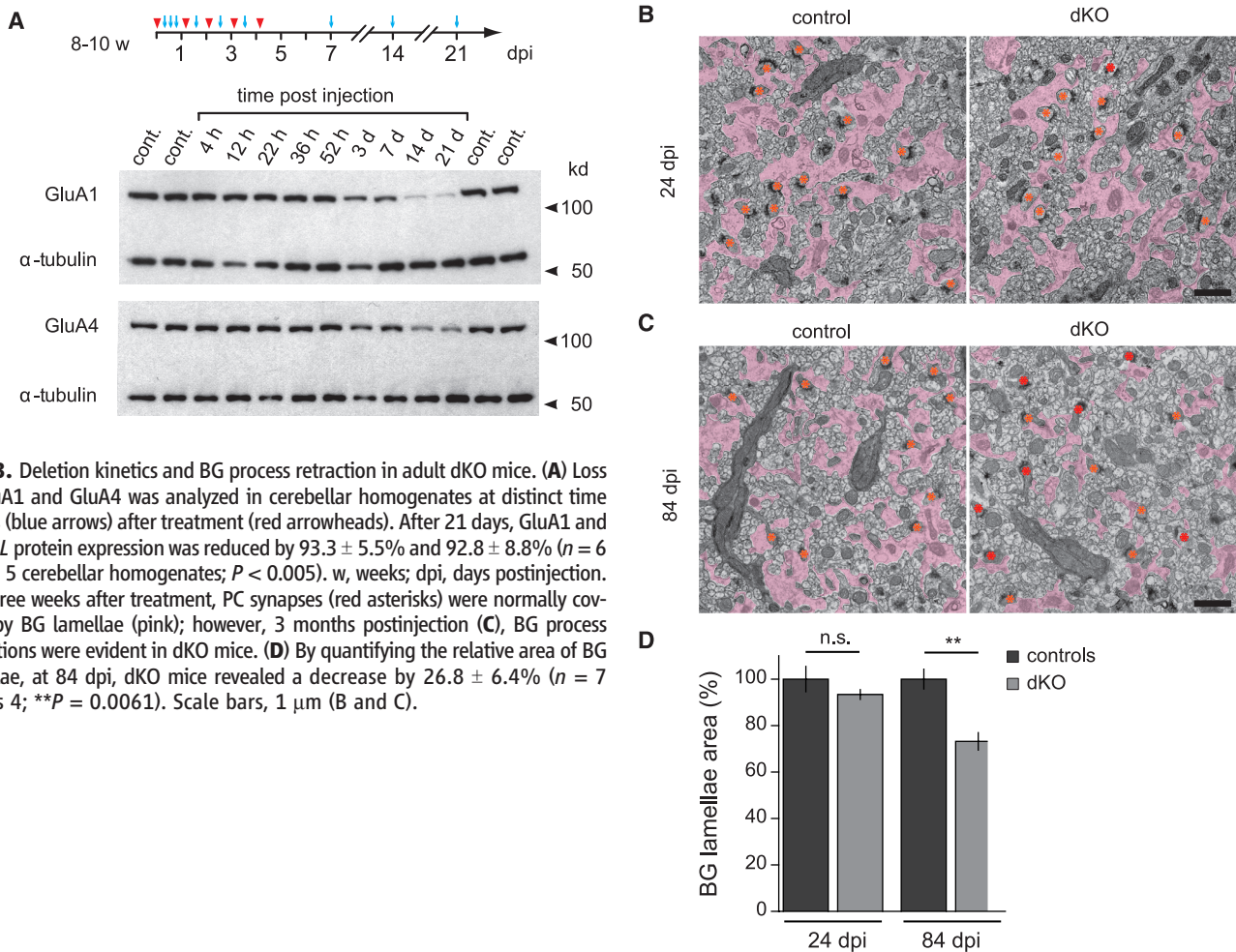


Fig. 3. Deletion kinetics and BG process retraction in adult dKO mice. **(A)** Loss of GluA1 and GluA4 was analyzed in cerebellar homogenates at distinct time points (blue arrows) after treatment (red arrowheads). After 21 days, GluA1 and GluA4L protein expression was reduced by $93.3 \pm 5.5\%$ and $92.8 \pm 8.8\%$ ($n = 6$ versus 5 cerebellar homogenates; $P < 0.005$). w, weeks; dpi, days postinjection. **(B)** Three weeks after treatment, PC synapses (red asterisks) were normally covered by BG lamellae (pink); however, 3 months postinjection **(C)**, BG process retractions were evident in dKO mice. **(D)** By quantifying the relative area of BG lamellae, at 84 dpi, dKO mice revealed a decrease by $26.8 \pm 6.4\%$ ($n = 7$ versus 4; $**P = 0.0061$). Scale bars, 1 μm (B and C).

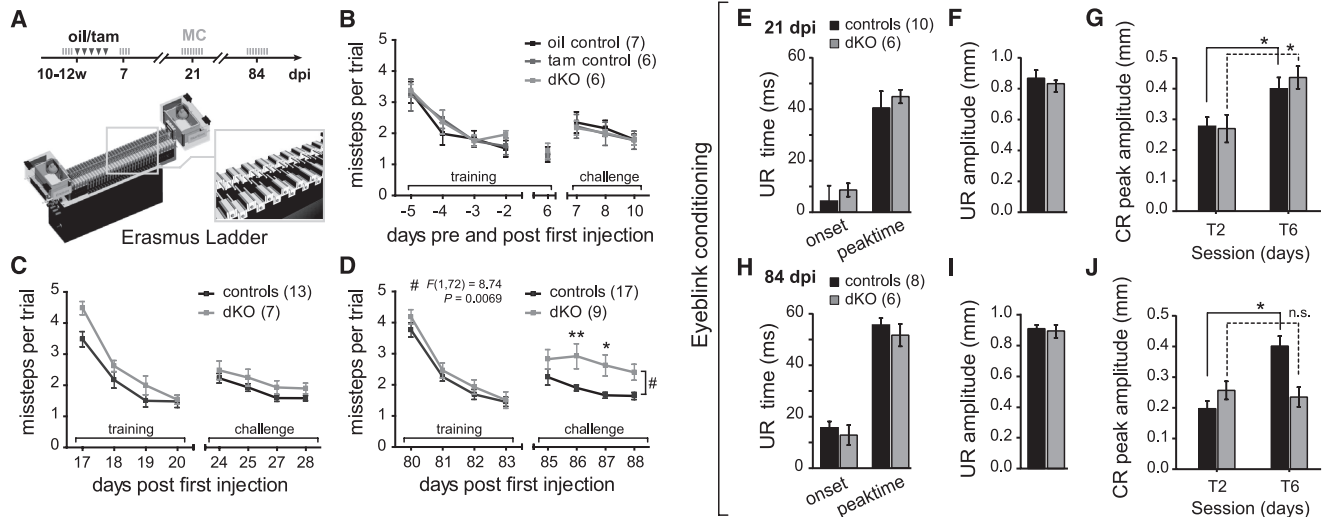


Fig. 4. Impaired fine motor coordination in mice lacking BG AMPARs. **(A)** At different time points before and/or after oil or tamoxifen treatment, motor coordination (MC) (i.e., average missteps per trial) was evaluated on the Erasmus Ladder (12, 17). **(B)** Tamoxifen treatment had no side effects on motor behavior, as control groups and dKO mice performed equally well directly after treatment. **(C)** At 3 to 4 weeks postinjections, dKO mice showed no differences in motor performance and motor learning. **(D)** At 3 months after treatment, dKO mice performed well during training sessions, but revealed more missteps when challenged with an abruptly elevated ladder rung

($F_{1,72} = 8.74$, $P = 0.0069$, two-way analysis of variance with Bonferroni's posttest). **(C and D)** Tam and oil controls were pooled. **(E to J)** Analysis of eyeblink conditioning data at 3 weeks (**E to G**) and 3 months (**H to J**) after treatment. dKO mice and littermate controls showed normal reflexive eyelid closure (UR) in response to the corneal air puff (**E, F and H, I**). The increase in amplitude of the conditioned response (CR) after consecutive training sessions was identical in controls and dKO mice at 24 dpi (**G**); however, at 84 dpi, dKO mice failed to increase their CR amplitude ($P > 0.4$) compared with controls ($P < 0.05$) (**J**). Data are represented as $\pm$ SEM.

of dKO mice (Fig. 4C). dKO mice did not reveal BG process retractions at that stage (see above). However, 3 months after injections, when BG process retraction was evident, dKO mice displayed significant deficits in their motor performance when challenged on the Erasmus Ladder (Fig. 4D); they showed more missteps per trial. There were no signs of learning deficits, in that dKO mice improved their performance during the training sessions, similar to controls. When we subjected the animals at the same postinjection periods to the eyeblink conditioning paradigm, we observed that timing and amplitude of unconditioned responses (URs) of dKO mice (Fig. 4, E, F, H, and I), as well as rate of memory acquisition or extinction of conditioned responses, (figs. S15 and S16) were indistinguishable from controls. However, at 3 months posttreatment, the amplitudes of their conditioned responses (extent of eyelid closures) were significantly lower than those of controls after consecutive training sessions (Fig. 4J). Retraction of BG processes from PC synapses may impair timing of PC firing (with millisecond precision), which, in turn, would affect the output of cerebellar nuclei neurons and, thus, conditioned behavior (20, 21).

We addressed the role of BG AMPARs on cerebellar function by generating conditional

AMPA mutants where both GluA1 and GluA4 subunits were efficiently ablated in young and adult mice. We revealed that AMPAR signaling of BG cells contributes to the structural and functional integrity of the cerebellar network. Our results provide *in vivo* evidence that BG AMPARs play an important role in the fine-tuning of neuronal processing, which is crucial for a fast and precise control of complex motor behaviors.

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Supplementary Materials

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The Pulvinar Regulates Information Transmission Between Cortical Areas Based on Attention Demands

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Selective attention mechanisms route behaviorally relevant information through large-scale cortical networks. Although evidence suggests that populations of cortical neurons synchronize their activity to preferentially transmit information about attentional priorities, it is unclear how cortical synchrony across a network is accomplished. Based on its anatomical connectivity with the cortex, we hypothesized that the pulvinar, a thalamic nucleus, regulates cortical synchrony. We mapped pulvino-cortical networks within the visual system, using diffusion tensor imaging, and simultaneously recorded spikes and field potentials from these interconnected network sites in monkeys performing a visuospatial attention task. The pulvinar synchronized activity between interconnected cortical areas according to attentional allocation, suggesting a critical role for the thalamus not only in attentional selection but more generally in regulating information transmission across the visual cortex.

The limited capacity of the visual system does not permit simultaneous processing of all information from our cluttered environment in detail. Selective attention helps overcome this limitation by preferentially routing behaviorally relevant information across the vi-

sual system. Simultaneous neural recordings from two cortical areas have suggested that this selective routing depends on the degree of synchrony between neuronal groups in each cortical area (1–4). However, it is unclear how different cortical areas synchronize their activity. Although direct interaction between two cortical areas may give rise to their synchrony, an alternative possibility is that a third area, connected to both of them, mediates cortical synchronization.

Higher-order thalamic nuclei, such as the pulvinar, predominantly receive input from the cortex rather than the periphery, and their output

strongly influences cortical activity in *in vitro* experiments (5). Because directly connected cortical areas are also indirectly connected via the pulvinar (fig. S1), the pulvinar is ideally positioned to synchronize activity across the visual cortex (6–8). However, little is known about the functional role of these cortico-pulvino-cortical loops. Selective attention modulates the magnitude of response of macaque pulvinar neurons (9, 10), and both humans and macaques with pulvinar lesions commonly have attentional deficits (11, 12). We therefore hypothesized that the pulvinar increases synchrony between sequential processing stages across the visual cortex during selective attention.

Information transmitted along the ventral visual cortical pathway is sequentially processed in interconnected areas V4 and the temporo-occipital area (TEO). We simultaneously recorded neural activity in macaques in the pulvinar, V4, and TEO during 51 recording sessions (13). Spike trains and local field potentials (LFPs) were recorded in each area from neurons with overlapping receptive fields (RFs). Monkeys performed a variant of the Eriksen flanker task, in which a spatial cue signals the location of a subsequent target flanked by distracter stimuli (target detection >80% accuracy overall; Fig. 1A). Because directly connected cortical areas such as V4 and TEO only connect with restricted but overlapping zones in the pulvinar (8, 14), we used diffusion tensor imaging (DTI) to ensure that electrodes targeted interconnected pulvino-cortical sites.

We performed probabilistic tractography on DTI data for each monkey to map probable

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connections between the pulvinar, V4, and TEO. We identified pulvinar zones connected with V4 (yellow) and TEO (red) and delineated the region of overlap (green) that the V4-pulvinar-TEO pathway probably traverses (Fig. 1, B and C). V4 and TEO predominantly connected to the ventral pulvinar, and there was substantial overlap between V4 and TEO projection zones in the pulvinar, with the TEO projection zone extending more caudally, consistent with previous anatomical tracer work (8, 14). However, probabilistic tractography data had the advantage of delineating projection zones specific to individual monkeys, which cannot be precisely ascribed on the basis of published tracer data. Guided by our structural connectivity maps, we positioned electrodes in the appropriate projection zones and verified their location therein by taking structural scans of each electrode track (fig. S7). We performed additional tractography analyses between voxels containing electrode tips to show probable paths running directly between recording sites (Fig. 1, D to F).

If the pulvinar plays an important role in selective attention, then pulvinar neurons should signal where the monkey attends in our flanker task. Figure 2, A and B, show the population activity of pulvinar neurons aligned to the cue and target onsets, respectively. Pulvinar neurons responded robustly to the cue in their RF. While the monkey maintained attention at the RF location, there remained a small but significant increase in pulvinar activity across the delay period (Student's *t* test, $P < 0.05$). When a stimulus later appeared in the RF, pulvinar neurons showed a significantly greater response when the monkey attended to the RF location rather than outside the RF (in the opposite visual hemifield; *t* test, $P < 0.05$). These results are consistent with previously reported attention-enhanced pulvinar responses to visual stimuli (10), and they additionally show that the attentional locus is represented by the pulvinar spike rate throughout the delay period when no stimuli are present.

We further tested whether attention influenced pulvinar spike timing, specifically the synchrony between pulvinar neurons, by calculating the degree of synchrony between spike times and the LFP, or spike-field coherence. For spectral analyses, we largely focused on the delay period after the cue-evoked response until the array onset, because not only did the monkey maintain spatial attention during this interval, but the data in each session generally satisfied methodological assumptions of stationarity as well. Figure S2, A and B, show a typical session in which pulvinar spike-field coherence increased immediately after the cue appeared in the RF, predominantly in the alpha-frequency range. While the monkey attended to the RF location, the spike-field coherence remained significantly elevated throughout the delay period until target presentation (*t* test, $P < 0.05$). When the cue appeared outside the RF (fig. S2, C and D), drawing the monkey's attention away from the RF, there was much weaker

spike-field coherence. At the population level, there was significantly greater spike-field coherence in the 8- to 15-Hz range (alpha band) during the delay period, until target presentation when the monkey attended to the RF location rather than outside the RF (Holm's controlled *t* tests, $P < 0.05$; Fig. 2C). Because synchronized thalamic output provides increased drive to the cortex in anesthetized animals (15, 16), increased synchrony of pulvinar neurons may be an effective means to influence the visual cortex during selective attention.

We next aimed to establish that selective attention increased synchrony between cortical areas (1–3). We calculated the coherence between V4 and TEO LFPs, which measures the synchrony between oscillatory processes in the two areas, as a function of oscillation frequency. Attention generally increased coherence between V4 and TEO LFPs in two frequency bands. There was significantly increased mean coherence in the 8- to 15-Hz range, as well as a smaller but significant increase in the 30- to 60-Hz range (gamma band) across the population (Holm's controlled *t* tests, $P < 0.05$) during the delay period until target presentation (Fig. 3A).

Because low-frequency oscillations modulate higher-frequency oscillations (17–19), we tested

whether attention increased cross-frequency coupling between alpha and gamma oscillations within V4 and TEO. To measure cross-frequency coupling, we calculated the synchronization index between cortical alpha oscillations and the gamma power envelope. Across the population, there was a significantly greater synchronization index for V4 and TEO during the delay period, when attention was directed to the RF location rather than outside the RF (sign tests, $P < 0.05$; fig. S3, A and B), suggesting that alpha oscillations contributed to the attention effect on gamma frequencies.

If the pulvinar interacts with the cortex during attentional processing, then attention should also modulate pulvino-cortical synchrony. Across the population, there was significantly greater alpha-band coherence between the pulvinar LFP and V4 LFP (Fig. 3B), as well as between the pulvinar LFP and TEO LFP (Fig. 3C) during the delay period until target presentation, when the monkey attended to the RF location rather than outside the RF (Holm's controlled *t* tests, $P < 0.05$). This result is consistent with previous reports of synchrony between the cat lateral posterior-pulvinar complex and visual cortex (20, 21). Pulvinar spike trains also synchronized with cortical LFPs. Figure 3, D and E, respectively

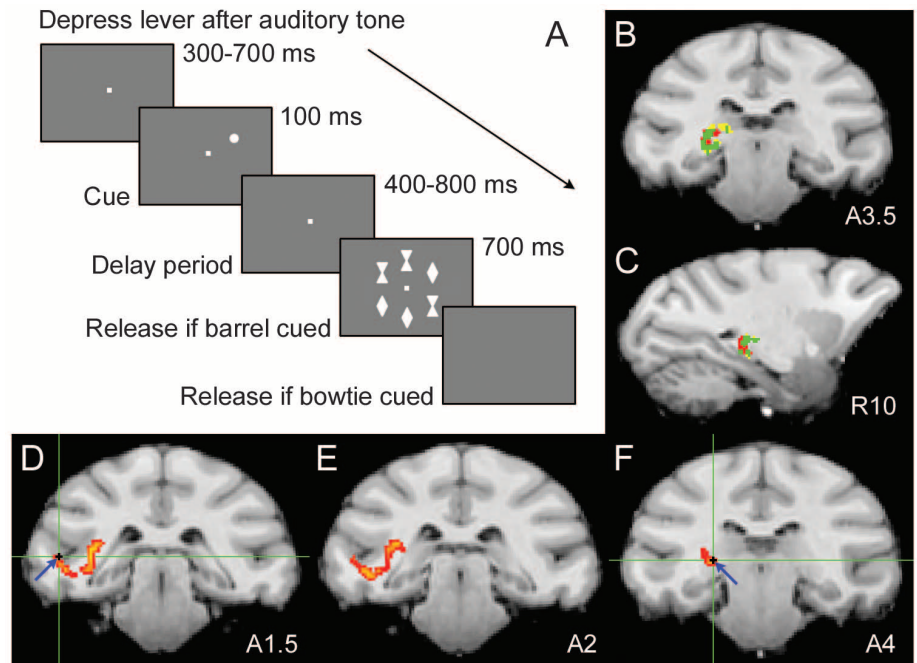


Fig. 1. DTI-defined pulvino-cortical network probed with spatial attention task. (A) We simultaneously recorded from the pulvinar, V4, and TEO of monkeys performing a flanker task. Monkeys maintained fixation throughout the trials while we manipulated their locus of attention. The monkeys' attention was drawn to the location of a cue, which randomly appeared at one of six locations. The cue signaled the location of the target in the subsequent array of six stimuli. To receive a juice reward, monkeys immediately released the lever after the onset of a barrel-shaped target or after the disappearance of the stimulus array for a bowtie-shaped target. (B) Coronal and (C) sagittal slices containing pulvinar voxels with a high probability of connection with V4 (yellow), TEO (red), or both (green). A3.5, 3.5 mm anterior to the interaural line; R10, 10 mm to the right of the midsagittal line. (D to F) Sequential coronal slices showing probable paths (yellow-red) between electrode tips [blue arrows and green cross-hairs in (D) and (F)] in TEO (D) and pulvinar (F) for one session.

show attention effects aligned to cue and target onsets for a typical session. Attention significantly increased coherence between pulvinar spikes and V4 LFP after the cue appeared in the RF until target presentation, predominantly in the 8- to 15-Hz range (t test, $P < 0.05$). There was much weaker coherence after the cue had drawn attention away from the RF (Fig. 3, F and G). We obtained similar attention effects on the co-

herence between pulvinar spikes and TEO LFP (fig. S4). Across the population, spatial attention significantly increased the coherence between pulvinar spikes and V4 LFP (Fig. 3H), as well as between pulvinar spikes and TEO LFP (Fig. 3I), predominantly in the 8- to 15-Hz range, throughout the delay period until target presentation (Holm's controlled t tests, $P < 0.05$). These findings support the idea that the pulvinar is part of

the brain's attention network (9, 12, 22–24) and that it uses the alpha band as a fundamental operating mode.

To determine the direction of pulvino-cortical interactions, we calculated the conditional Granger causality in the frequency domain for the connections between the pulvinar, V4, and TEO. Conditional Granger causality measures the influence that one area (e.g., the pulvinar) has on a second area (e.g., TEO), accounting for the influence of other areas (e.g., V4). This allowed us to dissect contributions from each connection and thus test our overall hypothesis that the pulvinar modulates cortical synchrony according to attentional demands. The pulvinar influenced oscillatory activity in both V4 and TEO when the monkey attended to the RF location. Figure 4, A and B, show that pulvinar influence on alpha activity in V4 significantly increased ($P < 0.05$) after the cue and continued until target presentation. There was much weaker pulvinar influence on V4 when the cue had drawn attention away from the RF (Fig. 4, C and D). Across the population, pulvinar influence on V4 (Fig. 4E; Holm's controlled t tests, $P < 0.05$) and on TEO (Fig. 4F; Holm's controlled t tests, $P < 0.05$) in the alpha-frequency range during the delay period was significantly greater with attention at the RF location than outside the RF. Pulvinar influence on alpha activity in both V4 and TEO correlated with the attentional modulation of synchrony between V4 and TEO in the same frequency range (Fig. 3A), suggesting that the pulvinar regulated alpha synchrony between cortical areas according to attention allocation.

In contrast to pulvino-cortical influences, direct cortico-cortical influences during the delay period were weak. Figure 4, G and H, show the population conditional Granger causality spectra for V4's influence on TEO and TEO's influence on V4, respectively. Spatial attention did not significantly change the weak influence of V4 on TEO, nor the weak influence of TEO on V4, during the delay period (t tests, $P > 0.05$). However, there was evidence consistent with strong cortico-cortical influences during visual stimulation (fig. S5). These results suggest that the maintenance of attention in the absence of visual stimulation depended on pulvino-cortical interactions (supplementary materials text) rather than direct cortico-cortical interactions.

Our results show that the pulvinar modulates the synchrony between cortical areas according to the locus of attention. The pulvinar predominantly influenced cortical alpha oscillations, consistent with another thalamic nucleus, the lateral geniculate nucleus, driving occipital alpha rhythms (25). Evidence suggests that the rhythmic excitability of alpha oscillations gates visual events, with the phase of alpha oscillations being critical for the transmission of visual information (26–28). Thus, the pulvinar, by synchronizing distributed patches of cortical alpha activity, can selectively facilitate transmission of information about attentional priorities across the cortex. Because pulvinar-

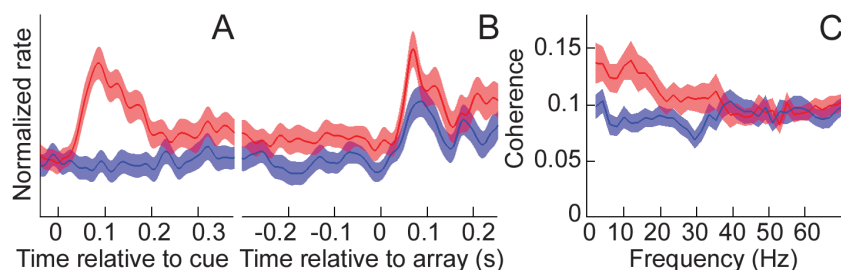


Fig. 2. Attention-modulated pulvinar spike rate and spike timing. Population activity ($\pm$ SE) aligned to (A) cue and (B) target onset is shown as the mean of 51 pulvinar cells. In (B), the preferred stimulus (barrel or bowtie) appeared at the RF, flanked by congruent distracters. (C) Population average of the transformed spike-field coherence in the pulvinar, calculated in the 300-ms window before target onset. Red, attention at the RF; blue, attention away from the RF.

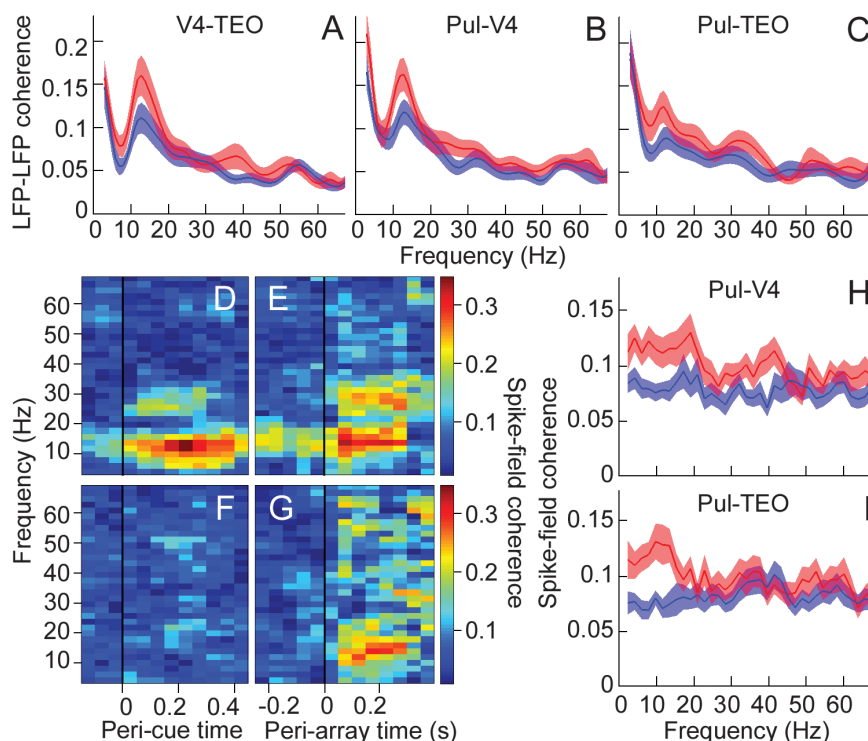
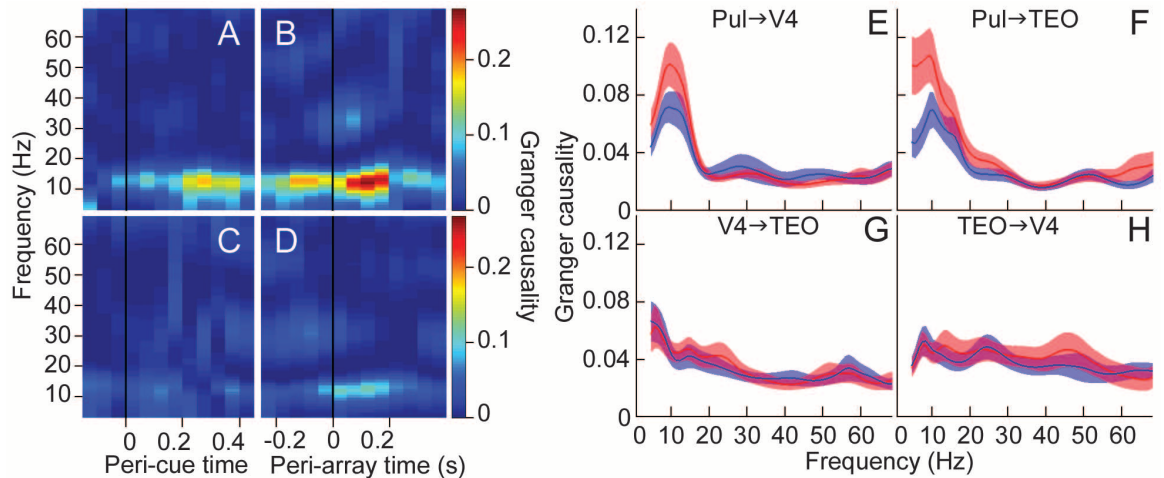


Fig. 3. Attention-modulated neural synchrony in pulvino-cortical networks. The population average of the transformed coherence between LFPs in (A) V4 and TEO, (B) pulvinar and V4, and (C) pulvinar and TEO, calculated in the 200-ms window before target onset, is shown. (D to G) Coherence (color-coded) between pulvinar spikes and V4 LFP for one session, calculated in successive 300-ms windows with a step size of 50 ms. (D) Cue and (E) target at the RF. (F) Cue and (G) target away from the RF. The same stimuli were presented in (E) and (G). The window immediately before the vertical black line in (E) and (G) represents the coherence 0 to 300 ms before target onset. The population average of the transformed coherence between (H) pulvinar spikes and V4 LFP and (I) pulvinar spikes and TEO LFP, calculated in the 300-ms window before target onset, is shown. Red, attention at the RF; blue, attention away from the RF.

Fig. 4. The pulvinar causally influenced cortical synchrony. (A to D) Conditional Granger causality (color-coded) from the pulvinar to V4 (accounting for TEO) for one session, calculated in successive 200-ms windows with a step size of 50 ms. (A) Cue and (B) target at the RF. (C) Cue and (D) target away from the RF. The same stimuli were presented in (B) and (D). The window immediately before the vertical black line in (B) and (D) represents the Granger causality 0 to 200 ms before target onset. The population average of the conditional Granger causality for (E) pulvinar influence on V4, (F) pulvinar influence on TEO, (G) V4 influence on TEO, and (H) TEO influence on V4, calculated in the 200-ms window before target onset, is shown. Red, attention at the RF; blue, attention away from the RF.



controlled alpha activity modulated gamma activity in the cortex through cross-frequency coupling, pulvinar influence on cortical synchrony extends to frequencies higher than the alpha frequency.

Pulvinar control of cortical processing challenges the common conceptualizing of cognitive functions as being restricted to the cortex. Pulvino-cortical influences dominated during the delay period, suggesting that internal processes such as the maintenance of attention in expectation of visual stimuli and short-term memory rely heavily on pulvino-cortical interactions. Pulvinar regulation of alpha activity is consistent with the important role ascribed to alpha oscillations in these internal processes (26, 29).

The prevailing view that information about our visual environment is transmitted through a network of cortical areas for detailed processing needs to be revised by considering extensive pulvino-cortical loops that regulate the information transmitted between each cortical stage of visual processing. Because of common cellular mechanisms and thalamo-cortical connectivity principles across sensorimotor domains, a general function of higher-order thalamic nuclei may be the regulation of cortical synchrony to selectively route information across the cortex.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S7

References (30–59)

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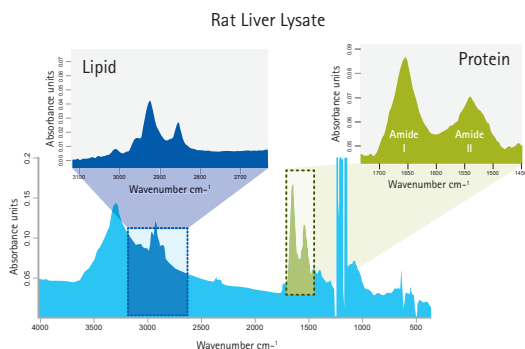
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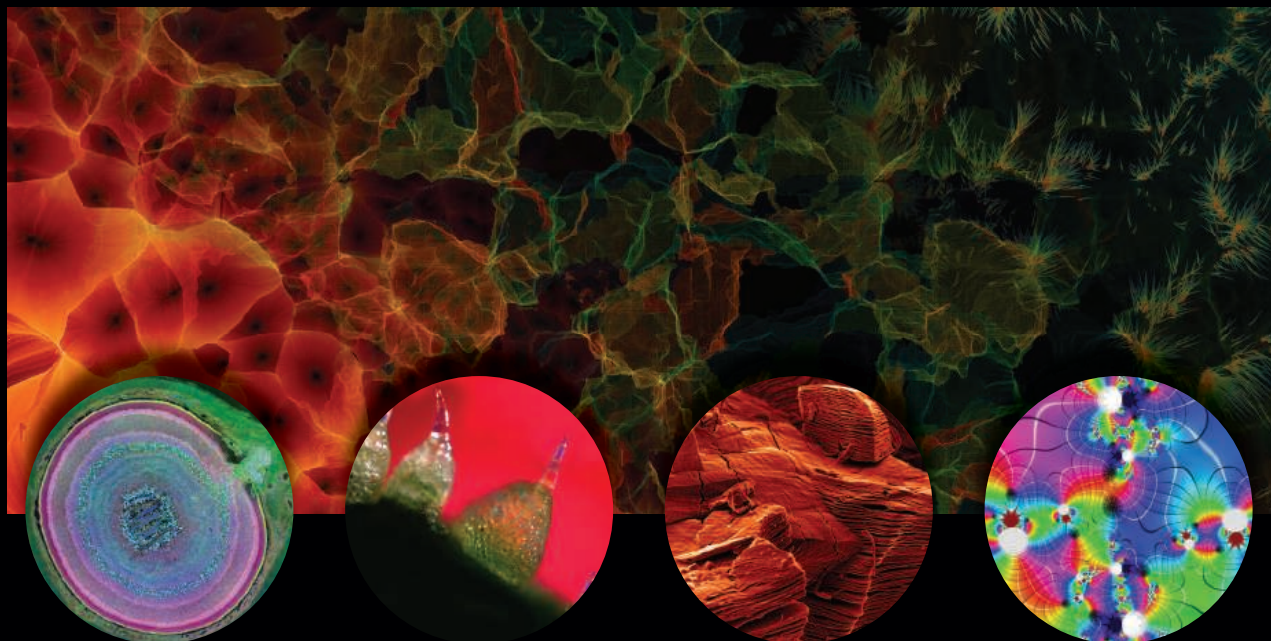


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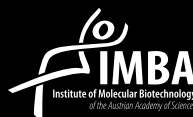
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The new C1 Single-Cell AutoPrep System enables researchers to isolate cells, extract RNA, and then reverse transcribe, and pre-amplify mRNA transcripts automatically to enable detection and analysis of cell activity. The C1 System workflow is initiated by loading a sample of cells in solution into the C1 microfluidic chip in a single pipetting step, then directing the C1 System to rapidly and automatically isolate up to 96 individual cells into individual chambers for preparation. After loading, researchers can choose an “in-process” quality control checkpoint to verify the number of captured cells and distinguish live from dead cells to preserve data integrity. The workflow then proceeds with a rapid “on-chip” cell lysis without RNA purification, reverse transcription, and preamplification without hands-on reagent mixing and sample transfer. The final preamplified cDNA product is thereafter harvested to collection wells for transfer to the BioMark HD System for quantitative polymerase chain reaction analysis.

Fluidigm

For info: 866-358-4354 | www.fluidigm.com

AUTOMATED SAMPLE RETRIEVAL

The newly launched arctic -20°C/-80°C high-density, low-cost storage unit holds up to 95,000 0.5 mL samples within a small footprint, making it ideal for space-restricted laboratories. It combines secure and safe sample tracking with automated sample retrieval, removing the loss of samples associated with manual methods. Handpicking is replaced with presorted, cherry-picked tubes delivered from store within 60 seconds. As a result, users can be confident in the integrity of their samples. arctic was built to offer the same quality and robustness as the well-known comPOUND -20°C modular sample storage system.

TTP Labtech Limited

For info: +44-(0)-1763-262626 | www.ttplabtech.com

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Micronic

For info: +31-320-277070 | www.micronic.com

MASS SPEC SAMPLE PREP

The AxION DSA system represents a technological breakthrough that eliminates sample preparation steps in addition to the need for front-end gas or liquid chromatography separation, as samples can be directly introduced to a mass spectrometer. This allows for faster and greatly simplified analysis, helping lead to faster decisions. With the AxION DSA system, sample prep time is now reduced to 25 seconds—down from 25 minutes. Designed to introduce samples to the AxION Time-of-Flight (TOF) mass spectrometer, the complete integrated system allows for the direct analysis of liquid, solid and gas samples. The system was designed with productivity and flexibility in mind, providing for sample analysis in seconds and a seamless switch from a DSA to a liquid chromatography system in less than two minutes. The integrated AxION DSA system is entirely enclosed to prevent atmospheric contamination of samples, ensuring exceptional sensitivity, and fully automated for fast data acquisition.

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With its historic attractions, vibrant arts and culture scene, Boston ranks among the world's most popular travel destinations. Special rates for meeting attendees are available at hotels in close proximity to the meeting and Boston attractions. The AAAS Annual Meeting registration and hotel online reservation system will go live on 10 August 2012.

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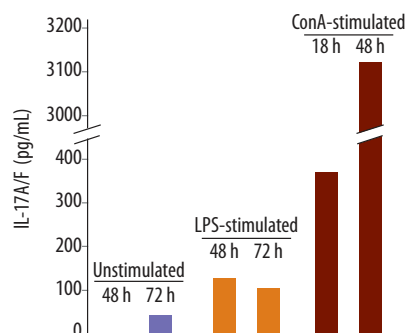
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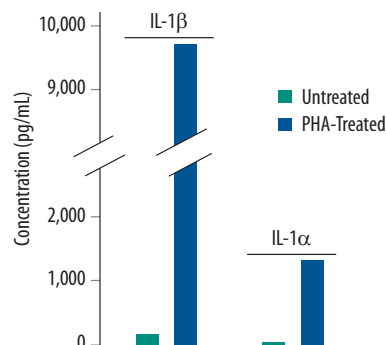
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From the journal *Science*



EDITOR-IN-CHIEF SCIENCE

The American Association for the Advancement of Science (AAAS), publisher of the international journal *Science*, has initiated a search for a new **Editor-in-Chief**. One of the premier journals in the world, *Science* is published weekly and includes reviews and reports of research across the spectrum of science and engineering, with an emphasis on those with cross-disciplinary impact and interest. The journal also serves as a forum for the presentation and discussion of the latest news and other issues surrounding science, with particular emphasis on the interactions among science, technology, government, and society.

In selecting an Editor-in-Chief, the Board of Directors will give weight to evidence of significant achievement in scientific research, editorial experience and creativity, awareness of leading trends broadly in science and scientific communication, and managerial abilities.

Applications or nominations should be accompanied by complete curriculum vitae, including a list of refereed publications, and should be sent to: **Gretchen Seiler, Executive Secretary, Search Committee, 1200 New York Avenue, NW, Washington, DC 20005**. Salary is negotiable based on qualifications and experience. Application materials should be sent by September 15, 2012.

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Faculty Opening for Division Chief July, 2012

The Department of Radiation Oncology at UT Southwestern Medical Center seeks an exceptional senior scientist to head the department's division of Molecular Radiation Biology. Applicants must have a Ph.D. or M.D./Ph.D. and a vigorous and ambitious independent research program in an area of cancer biology that is related to radiation oncology, as well as grant funding and a significant record of peer-reviewed publication. The program should complement and expand existing research strengths within the division, which includes molecular oncology, cell signaling, hypoxia and the DNA damage response. Candidates should have a successful record as the manager of a productive research team and an enthusiasm for promoting collaboration within a multidisciplinary environment.

The Division of Molecular Radiation Biology currently has 60 employees, including seven senior Ph.D. investigators and three M.D./Ph.D. translational researchers as well as other junior research faculty, postdoc and graduate student trainees, and lab technicians. There is also significant dedicated administrative support staff. The division has approximately 10,000 square feet of contiguous lab space, including 6 large labs and core space for tissue culture, microscopy, and other shared resources.

The appointment will be at the level of Professor, with tenure (contingent upon approval by the institutional promotion and tenure committee). A generous start up package will include substantial lab space and funding for equipment or faculty/staff/trainees. Of particular note, the Cancer Prevention and Research Institute of Texas (CPRIT) has awarded over \$106 million in grants to investigators at UT Southwestern, including funding opportunities for recruitment of pre-eminent cancer investigators.

Applicants should email their curriculum vitae, the names of three references, and a brief description of their research goals to the attention of **Dr. Hak Choy** at Hak.Choi@utsouthwestern.edu.

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Research Scientist Pharmacology

Bayer Pharma AG, Berlin, Germany

Job description As a research scientist for Bayer Pharma AG, Berlin, your tasks will involve characterizing novel drug candidates as potential treatment option for oncology patients. You will establish state of the art in vitro assays to characterize the mode of action of drug candidates and use them in the characterization, optimization and selection processes. Your expertise is strong in establishing innovative animal models to support the drug candidate development. Your assignments also include the scientific planning, evaluation, interpretation and presentation of experimental studies, as well as the collaboration in interdisciplinary project groups.

Your qualifications Your profile comprises a university degree in Biology, Biochemistry, Pharmacy or Veterinary Medicine with a PhD in the field of oncology, veterinary sciences or pharmacology. To be successful in this role, a very good knowledge, and strong hands-on experience, in the field of in vitro pharmacology and the performance of tumor experiments in vivo is essential. You should have several years of professional experience in the field of oncology or related discipline, preferably in an industrial setting. Knowledge and expertise in the areas of signal transduction pathways, immunotherapy, tumor metabolism or in vivo imaging would be an advantage. Outstanding written and verbal English skills are required, basic German knowledge is helpful. The ability to deal routinely with conventional IT applications is required. Your profile is rounded off by the ability to work in a team as well as independently, a sense of responsibility, the ability to work under pressure, organizational talent and the expertise to instruct and train staff.

Your application We offer a competitive salary in an international environment as well as excellent opportunities for professional and personal development. If your background and personal experience fits this profile, please send us your complete application at www.myBayerjob.de (Reference Code 0000039864), submitting a cover letter, your CV and references.

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UNIVERSITY OF
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FIAT

**Faculty of Health and Life Sciences
Institute of Translational Medicine
Department of Molecular and Clinical Pharmacology
and MRC Centre for Drug Safety Science**

The University of Liverpool invites applications for a number of Chair posts based in the Department of Molecular and Clinical Pharmacology and prestigious MRC Centre for Drug Safety Science. The MRC Centre provides fundamental mechanistic insight into clinically important adverse drug reactions to allow for safer drug design and more informed patient care.

These posts are part of the University's continued and significant commitment to the MRC Centre. You must be an outstanding individual with established independent research programmes able to take senior strategic roles in the MRC Centre as it expands. Candidates from the Pharmaceutical Industry will also be considered. Attractive support packages will be available together with high quality laboratory research space and an opportunity to develop further refurbished research laboratories.

Chair in Molecular Pharmacology/Toxicology

Salary Negotiable

To develop any area of Molecular Pharmacology or Molecular Toxicology relevant to the goals and remit of the MRC Centre. **Job Ref: A-576899/S**

Chair in Cancer Pharmacology

Salary Negotiable

To develop Cancer Pharmacology and support the development of novel therapeutic agents with a particular focus on safe drug design. **Job Ref: A-580278/S**

Chair in Pharmacometrics

Salary Negotiable

To strengthen the quantitative pharmacology work of the Centre, we seek a specialist in any of the following fields: pharmacokinetics and pharmacodynamic modelling or pharmacoepidemiology. **Job Ref: A-576898/S**

Chair in Systems Pharmacology

Salary Negotiable

We seek a Systems Biologist or Bioinformatician to develop Systems Pharmacology at the University. **Job Ref: A-580277/S**

Closing date for all posts: 3 September 2012

For full details, or to request an application pack, visit www.liv.ac.uk/working/job_vacancies/ or e-mail jobs@liv.ac.uk Please quote job ref in all enquiries.

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ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Assistant Professor of Cellular Biochemistry Assistant Professor of Systems Biology

The Department of Biology (www.biol.ethz.ch) at ETH Zurich invites applications for above-mentioned Assistant Professorships (non-tenure track).

The Department of Biology offers outstanding scientific opportunities to participate in interdisciplinary research projects, including close interactions with the Swiss Initiative in Systems Biology (SystemsX.ch), the Functional Genomics Center Zurich, and the light- and electron microscopy facilities at ETH Zurich. An interactive, state-of-the-art research environment is provided at the campus Hoenggerberg, with colleagues in computer science, chemistry and physics both at ETH Zurich and University of Zurich.

Research programmes in **Cellular Biochemistry** should be directed towards molecular mechanisms underlying the dynamic organisation of cellular processes and cell architecture with a preferred emphasis on membrane dynamics and lipid biology. Potential topics include, but are not limited to cell growth and division, intracellular transport, and organelle organisation and inheritance. The professorship will ideally be located in the Institute of Biochemistry, taking advantage of its strengths in light microscopy.

Research in **Systems Biology** should focus on cellular systems and processes as integrated networks of interacting molecules. The research programme should be directed to the dynamics of uni- or multicellular systems by applying or developing quantitative methods that may include omics techniques, live imaging or single cell analyses. The professorship will be part of the Institute of Molecular Systems Biology.

The successful candidates will be expected to establish and lead an independent and internationally competitive research group that attracts external funding, with the support of the joint mentoring programme between the Department and the hosting Institutes. In addition, they will actively contribute to the research environment in the Department, and participate in the teaching of graduate and undergraduate students. They will contribute to the ongoing efforts to increase teaching of quantitative approaches in biology. In particular, the candidates will participate in the Bachelor and Master curriculum in Systems Biology and Cellular Biochemistry. The new professors will be expected to teach undergraduate level courses (German or English) and graduate level courses (English).

The assistant professorships have been established to promote the careers of younger scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period.

Your application should include your curriculum vitae, a list of publications, a statement of your research and teaching interests, and the names of at least three referees. The letter of application should be addressed to the **President of ETH Zurich, Prof. Dr. Ralph Eichler. The closing date for applications is 30 September 2012.** ETH Zurich is an equal opportunity and affirmative action employer. In order to increase the number of women in leading academic positions, we specifically encourage women to apply. ETH Zurich is further responsive to the needs of dual career couples and qualifies as a family friendly employer. **Please apply online at www.facultyaffairs.ethz.ch.**

Molecular Biomarker Scientist

Who we are

At Roche, 80,000 people across 150 countries are pushing back the frontiers of healthcare. Working together, we've become one of the world's leading research-focused health-care groups. Our success is built on innovation, curiosity and diversity.

The headquarters in Basel is one of Roche's largest sites, over 8,000 people from approximately 80 countries work at Roche Basel. Favored by its geographic location in the heart of Europe, the Basel area is one of the most dynamic economic regions in Switzerland – a great place to live and work.

The Position

As Molecular Biomarker Scientist you are part of the Discovery group in the Neuroscience Department at the Roche Headquarters in Basel. The core responsibility is to develop molecular biomarker strategy for projects starting at entry into human GLP, and to communicate this strategy to the corresponding Biomarker Experimental Medicine Leaders and Translational Medicine Leaders. In this position you will:

- assess the literature/science on genetics of CNS disorders and drug responders/non-responders to suggest new targets and patient stratification criteria
- work closely with BEMs to facilitate implementation of molecular biomarker strategy for individual projects
- perform and/or oversee analysis of genetic biomarker data from clinical trials and experimental medicine studies
- be encouraged to write project proposals to secure funding for Postdocs to support the Biomarker team

Who you are

You're someone who wants to influence your own development. You're looking for a company where you have the opportunity to pursue your interests across functions and geographies. Where a job title is not considered the final definition of who you are, but the starting point.

As the successful applicant you:

- have experience with high dimensional data analysis of genomic and proteomic data, clinical trial methodology, and biostatistical analysis of clinical trial data
- are comfortable analyzing large data sets
- have a PhD and postdoctoral research experience in molecular neuroscience, human genetics, or efficacy pharmacogenetics
- are proactive and work effectively with different groups and team members
- are fluent in English (spoken and written)

Job ID No.: 00400273

Contact HR: K. Arnold, Phone: +41 61 68 74476

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西北农林科技大学

Northwest A&F University

Academic Talents Recruitment

Northwest A&F University, Shaanxi, China

Northwest A&F University (NWAUFU) is one of the key universities in China supported by the Central Government under the "Project 985" and "Project 211". It is located in Yangling, Shaanxi Province, the birth place of Chinese agricultural civilization. Founded in 1934 as the first high education institution in modern agriculture and forestry, NWAUFU has achieved significant progresses and developments in the past 78 years. Now with its 23 colleges and departments, NWAUFU is the most comprehensive university in agriculture, forestry and hydro-science in China and one of the nation's premier research-oriented universities.

Recently, NWAUFU has established Special Professorships in the following academic areas and invites highly talented and motivated candidates to apply:

I. Academic areas with job openings

Maize genomics and molecular breeding,
Vegetable molecular breeding,
Tree genetics and breeding,
Animal molecular breeding,
Apple genomics,
Functional genomics of plant nutrition,
Crop functional genomics,
Soil chemistry,
Soil erosion prediction model,
Animal cell engineering and embryo engineering,
Pathogenic microorganisms of animals,
Plant stress biology,
Material cycling in watershed ecosystem,
Structural optimization and structure-function relationship of biologically active natural substances,
Food safety,
Theoretic and techniques of crop high-efficiency water use,
Hydrological modeling theory and methods,
Agricultural machinery and technology,
Forestry economic theory and policy,
Contemporary sociological theory and research methods.

II. Applicant Eligibility

- 2.1 Ph.D. degree in related disciplines. Under 45 years old for applicants in natural sciences and under 50 years old for those in social sciences.
- 2.2 Holding an assistant professor or more senior position for international applicants. A rank of full professor or equivalent for domestic applicants.

III. Benefits and Salary

- 3.1 Entitlement of full professor and supervisor of Ph.D. students.
- 3.2 A minimal startup funding of ¥3 million for candidates in natural sciences and ¥1 million for those in social science.
- 3.3 An apartment (150-180m²) and a ¥0.3 million household allowance will be provided. Ownership of the apartment will be transferred to the faculty members after serving the university for ten years.
- 3.4 In addition to regular salary and benefits, qualified applicants will be compensated with an annual allowance of ¥0.1-0.5 million. International candidates will receive a minimal annual salary of ¥0.6 million.
- 3.5 Spouse hiring will be accommodated.

IV. Application Procedure

- 4.1 Applicants should provide: An English version of curriculum vitae with lists of publications, research interests and professional titles and activities; A statement of research accomplishments and future teaching and research plan; PDF copies of academic transcripts, diploma and degree certificate, five representative papers or books and documents for funded projects, awards, patents and keynote speakers at international conferences, etc; Three recommendation letters with referrers' e-mails and telephone numbers.
- 4.2 NWAUFU will conduct a preliminary screen and invite eligible candidates to Yangling for an interview and campus visit. The selected candidates need to sign a contract with the university. See our Chinese ad at <http://rcb.nwsuaf.edu.cn/show.php?articleid=539>

V. Contact

Address: No. 3 Taicheng Road, Yangling, Shaanxi Province, 712100 China.
Contact Persons: SUN Ma, or ZHANG Pengfei
Tel: +86-29-87082855, 87082577, Fax: +86-29-87082855
E-mail: rencaike@nwsuaf.edu.cn; rencaiban@gmail.com.
The university's website: <http://www.nwsuaf.edu.cn>



INTERNATIONAL
VACCINE INSTITUTE

THE INTERNATIONAL VACCINE INSTITUTE
POSITION ANNOUNCEMENT

-Portfolio Management Director-

The International Vaccine Institute (IVI) is an independent international organization and focuses on research and development of new and improved vaccines for use in developing countries. The Institute is located in Seoul, Korea. For more information about IVI, please visit www.ivi.int.

IVI is looking for a Portfolio Management Director (PMD). The PMD will oversee the main programs of the Institute, as well as Business Development and Grant Management. In addition, the PMD will co-chair with the Chief Scientific Officer, a Review Committee that will internally monitor all of the Institute's research programs.

The roles and responsibilities of the PMD include:

- To ensure that each program is on target according to the milestones and budget.
- To ensure high quality and timely reporting to funders.
- To lead the Institute's strategic prioritization process with strategic planning.
- To develop Portfolio Management systems and processes.
- To be the Performance Leader for the Program Leaders, Business Development Manager, and Grant Manager.
- To recruit staff and build a team for the new Portfolio Management Unit.
- To identify and seek new sources of fundings.

The candidate should have a minimum of 15 years of experience in the vaccine industry or in product development partnerships and possess a solid understanding of vaccine R&D. A Ph.D. in relevant scientific disciplines is preferred but a successful and proven track record in portfolio management will also be considered. As the position involves working in a multicultural setting, the PMD must be a quick learner and nimbly adapt to fluidly changing circumstances. Proficiency in English is required.

Letters of interest along with current curriculum vitae should be received by **August 31, 2012** and should be submitted via IVI website www.ivi.int.

FRED HUTCHINSON
CANCER RESEARCH CENTER

VACCINE AND INFECTIOUS DISEASE DIVISION

FACULTY POSITION

The Vaccine and Infectious Disease Division (VIDD) at the Fred Hutchinson Cancer Research Center (FHCRC) is soliciting applications to fill a faculty position with an individual who has a doctoral degree and who is conducting innovative translational research on the connection between cancers and pathogenic or commensal microbes. Areas of research interest include pathogen discovery, oncogenic mechanisms of cancer-related pathogens, the role of commensal microbiota in cancer, microbe-specific immunology, or vaccine development. Candidates at any rank will be considered. Opportunities exist for joint (secondary) appointment in the Divisions of Clinical Research, Public Health Sciences or Human Biology at FHCRC, as well as an affiliate appointment at the University of Washington.

As one of five divisions within the FHCRC, VIDD focuses on a wide range of human pathogens. The Center's Seattle Cancer Care Alliance (SCCA) provides opportunities for translational research through access to patient populations with a wide variety of cancers and hematopoietic stem cell transplant recipients. Ongoing collaborations among the FHCRC, SCCA and the Uganda Cancer Institute provide rich and unique resources for translational research in HIV-related malignancies, especially Kaposi sarcoma and Burkitt lymphoma.

VIDD occupies state-of-the-art research laboratories on a beautiful lakeside campus. The Center offers outstanding shared resources, including computational biology, genomics, proteomics, and imaging facilities. The Center has active training programs for graduate students and postdoctoral fellows and offers exceptional opportunities for scientific interactions with other investigators in the Seattle area and abroad.

Salary DOE + excellent benefits. To assure consideration, candidates should apply by **October 31, 2012** by sending a curriculum vitae, a concise statement of research plans, and contact information for three (3) references, along with a cover letter to **Ms. Christina Wee, Faculty Affairs Office: viddfao@fhcrc.org**. Later applications may also be considered if the position is not yet filled.

*The Fred Hutchinson Cancer Research Center is an
Affirmative Action, Equal Opportunity Employer.*

THE UNIVERSITY of TENNESSEE
HEALTH SCIENCE CENTER

**The University of Tennessee College of Pharmacy
Pharmaceutical Sciences Faculty Position**

The Department of Pharmaceutical Sciences in the College of Pharmacy at the University of Tennessee Health Science Center in Memphis, TN, is seeking applications for a twelve-month full-time, state supported tenure-track faculty position at the full, associate, or assistant professor level. The successful candidate is expected to devote greater than a 60% effort to research. The applicant should have core expertise in medicinal chemistry, pharmacology, pharmaceuticals, chemical biology, biomedical engineering, structural biology or a related discipline focused on drug discovery and development. Candidates with a well-funded program in cancer, diabetes, antibiotics, drug delivery, nano-medicine, nano-technology, bio-imaging, or lipid research are highly encouraged to apply. The successful candidate is expected to have a Ph.D. or equivalent degree, active extramural support as principal investigator (e.g., National Institutes of Health funding), a commitment to excellence in teaching and excellent oral and written communication skills.

Applications will be processed until the position is filled. Please submit curriculum vitae, summary of research interests, contact information, and three letters of reference to: **Isaac Donkor, Ph.D. Professor and Vice Chair, Chair of Faculty Search Committee, Department of Pharmaceutical Sciences, 847 Monroe Ave, Suite 327, Memphis, TN 38163.**

The University of Tennessee Health Science Center is located in Memphis, TN, an economically vibrant center, with a metropolitan population of more than 1.3 million, reflecting the richness along the bluffs of the mighty Mississippi River. The College of Pharmacy is located in a new, 187,000 square-foot building on the Health Science Center complex.

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**Editor, The Lancet – ELSEVIER
Health Sciences : USA-NY-New York**

The Lancet seeks an Editor based in New York to join its team of physicians and scientists. You will be involved in all aspects of work for this weekly journal, including peer review, commissioning, and writing, with specific emphasis on strengthening *The Lancet's* reputation and influence in North America. In this role, you will provide a point of contact for researchers and clinicians in North America, and represent *The Lancet* family of journals externally and internally within Elsevier. You will have substantial responsibility to attract top quality research and to help develop and implement *The Lancet's* strategy for the region.

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Qualifications: In addition to a medical qualification or a Ph.D. in a health-related discipline, you should have research experience and a wide-ranging interest in all aspects of medicine. Sound editorial experience and a passion to write would be an advantage. To find out more, you can telephone **Dr. Bill Summerskill** at *The Lancet's* London office on +44 20 7424 4920. To apply, please send your CV and a cover letter to **b.summerskill@elsevier.com**, stating why you feel you are suitable for the job and your current salary details.

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Director, National Institute of General Medical Sciences, National Institutes of Health



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We are looking for applicants with senior level experience who have a commitment to excellence and the energy, enthusiasm, and innovative thinking necessary to lead a dynamic and diverse organization.

The successful candidate for this position will be appointed at a salary commensurate with his/her qualifications. Full Federal benefits will be provided including leave, health and life insurance, long-term care insurance, retirement, and savings plan (401k equivalent).

If you are ready for an exciting leadership opportunity, please see the detailed vacancy announcement at <http://www.jobs.nih.gov> (under Executive Jobs).

Applications will be reviewed starting October 1, 2012 and will be accepted until the position is filled.



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MEETINGS



EUROPEAN CONGRESS OF IMMUNOLOGY

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Book both **The European Congress of Immunology** and **4th European Veterinary Immunology Workshop** and benefit from a **10% discount** on both congresses. EVIW will be held from the **September 2-4, 2012** in Edinburgh.

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- EVIW is being held at the **Royal College of Surgeons, Edinburgh** – only one hour from Glasgow
- Both congresses offer a **rich scientific programme** focusing on current and future trends in immunology
- Your opportunity to **discover Scotland**

POSITIONS OPEN

ASSISTANT/ASSOCIATE PROFESSOR in Infectious Diseases

A scientist using multidisciplinary approaches to investigate Biosafety Level 3 (BSL3) organisms is sought for a tenure-track position at Montana State University in Bozeman. We are seeking an individual with a research program focused on BSL3 pathogens that pose a significant health risk to humans and animals. The new hire will be an Investigator in the Center for Zoonotic and Emerging Infectious Diseases ([website: http://www.montana.edu/cobre](http://www.montana.edu/cobre)). The position appointment is ~70% research, and includes a competitive institutional salary (nine months), and a generous startup package. Pathogen-containment facilities are in place and accommodate small (BSL3 & BSL-2) and large (ABSL-2) animal research. The Department has strong research programs in immunology and microbial pathogenesis and is housed in a state-of-the-art facility with core laboratories for flow cytometry, cell biology, and molecular sciences. A doctoral degree in a biomedical discipline, postdoctoral experience, and the potential to establish an independent competitive research program are required for the Assistant Professor level. For the Associate level, evidence of an established research program is required. A letter of application, curriculum vitae, and summary statement describing research plans and grant proposals should be sent, preferably electronically as a single PDF, to **e-mail: cherylj@montana.edu, Attn: Faculty Search**. Three letters of reference should be sent to the same e-mail address or to: **Chair, Search Committee, Department of Immunology and Infectious Diseases, PO Box 173610, Montana State University, Bozeman, MT 59717-3610**. Screening will begin September 1, 2012 and will continue until a suitable applicant is hired. For a full job description and additional information about our department, visit our **website: http://iid.montana.edu**. *ADA/Equal Opportunity/Affirmative Action/Veterans Preference.*

ASSISTANT PROFESSOR, TENURE-TRACK College of Saint Benedict/Saint John's University Biology, Genetics

The joint Biology Department of the College of Saint Benedict and Saint John's University is seeking to fill a full-time tenure track, assistant professor position starting August 2013. We seek a dynamic teacher-scholar with a strong commitment to teaching, service, and research in an undergraduate liberal arts environment. The successful candidate will have a Ph.D. and must be able to teach introductory biology, a course in genetics, and courses in the candidate's area of specialty. Although all candidates with genetic and genomic research interests will be considered, candidates whose research interests involve the application of genetic or genomic techniques to our extensive campus natural settings are especially encouraged to apply. Candidates should provide evidence of their potential to excel at teaching, develop a research program involving undergraduates, and compete for extramural funding.

The College of Saint Benedict, a liberal arts college for women, and Saint John's University, a liberal arts college for men, and are located four miles apart in Central Minnesota just outside metropolitan Street Cloud and 70 miles from Minneapolis. Both are Catholic colleges in the Benedictine tradition, which emphasize quality teaching and a commitment to intercultural learning. For further information, see **website: http://www.csbsju.edu**.

Visit our website for more information and to apply online at **website: http://employment.csbsju.edu**.

Women, individuals of diverse racial and cultural backgrounds, and persons with disabilities are encouraged to apply. College of Saint Benedict and Saint John's University are Affirmative Action/Equal Opportunity Employers.

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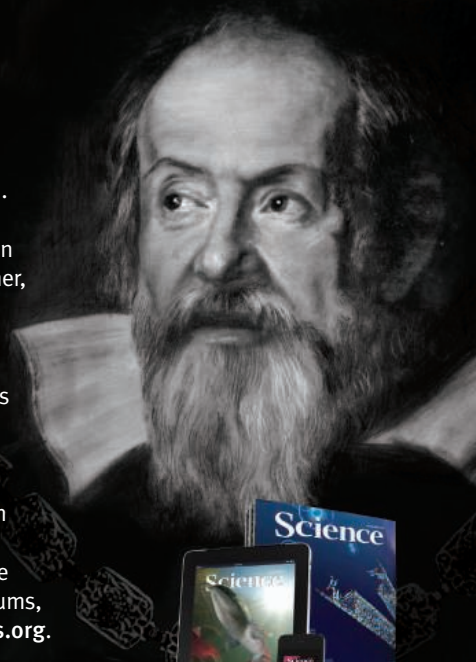


There's only one GALILEO GALILEI

Born in 1564, Galileo Galilei once contemplated a career in the priesthood. It's perhaps fortunate for science that upon the urging of his father, he instead decided to enroll at the University of Pisa. His career in science began with medicine and from there he subsequently went on to become a philosopher, physicist, mathematician, and astronomer, for which he is perhaps best known. His astronomical observations and subsequent improvements to telescopes built his reputation as a leading scientist of his time, but also led him to probe subject matter counter to prevailing dogma. His expressed views on the Earth's movement around the sun caused him to be declared suspect of heresy, which for some time led to a ban on the reprinting of his works.

Galileo's career changed science for all of us and he was without doubt a leading light in the scientific revolution, which is perhaps why Albert Einstein called him the father of modern science.

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Research Fellow Thymic Development

A postdoctoral position is now available for a highly motivated scientist to study thymic development involving plexin-semaphorin regulated migration of thymocytes. The successful candidate should have a PhD degree and be familiar with basic techniques such as animal handling, multicolor flow cytometry, multicolor confocal microscopy and cell biology. Research experience in immunology and strong background in T cell development/migration are essential, along with expertise in biochemistry/molecular biology.

If interested, please send your CV, a brief summary of research experience and names of three referees to: Ellis Reinherz, MD, Professor, Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Ave., Boston, MA 02215; Email: ellis_reinherz@dfci.harvard.edu.

Dana-Farber Cancer Institute is an Affirmative Action/Equal Opportunity Employer.



Yale University Faculty Position in Plant Ecology

The Department of Ecology and Evolutionary Biology at Yale University invites applications for a tenure-track assistant professor position in plant ecology, including interactions between plants and other organisms and biogeochemical cycles. We seek applicants that use creative approaches to address fundamental questions in ecology. We are particularly interested in applicants that have or would develop a strong field research program. A record of outstanding achievement and a promising research program are more important than the specific research area.

Interested candidates should submit a CV, three relevant reprints or manuscripts, brief research and teaching statements, and names and addresses of three potential evaluators online at <https://academicjobsonline.org/ajo/Yale/EEB>. The search will remain open until the position is filled. The review of applications will begin 1 September 2012.

Yale University is an Equal Opportunity/Affirmative Action Employer. Yale values diversity among its students, staff, and faculty and strongly welcomes applications from women and underrepresented minorities.



Research Microbiologist, GS-0403-12/13 Salary Range of \$68,809 to \$106,369

The Food Safety and Enteric Pathogens Research Unit in the National Animal Disease Center in Ames, Iowa is seeking a qualified Research Microbiologist to conduct culture based and metagenomics analysis of microbial communities in the turkey intestinal tract. The scientist develops alternatives to antibiotics for controlling intestinal colonization by the foodborne pathogen *Campylobacter*. NADC is the premier research institute within the USDA for studying diseases of large animals and has recently undergone a \$500 million upgrade of laboratory facilities. Resources for the position include flow cytometry, mass spectroscopy, laser capture microdissection microscopy, Roche FLX, Solexa-Illumina and AP3100 sequencers, an electron microscopy/histology facility, bioinformatics resources, and both conventional and gnotobiotic large animal support.

A Ph.D. in microbiology or a related field is required. Assigned research requires a professional knowledge of microbial genetics diversity, biochemistry, and pathogenesis; metagenomic analyses of microbial ecosystems. Contacts: Application procedure: Kim Grandon 515-337-7277. Scientific information: Thad Stanton@ars.usda.gov. For details and application directions, visit the website www.usajobs.gov and refer to announcement number ARS-X12E-0080. U.S. citizenship is required. Applications must be made between Aug. 20 and Sept. 28, 2012.

USDA/ARS is an Equal Opportunity Employer and Provider.

POSITIONS OPEN



ASSISTANT PROFESSOR Biological Chemistry The Johns Hopkins University School of Medicine

The Department of Biological Chemistry at The Johns Hopkins University School of Medicine invites applications for a new tenure-track faculty position at the Assistant Professor level. The Department is seeking candidates with an outstanding record in any area of biochemistry, cellular, or molecular biology and a commitment to excellence in research and teaching. Applicants should submit (preferably, as a single PDF file) curriculum vitae, list of publications, summary of research accomplishments, and a description of their future research plans by October 31, 2012. Electronic files should be sent to e-mail: bcfacultyrecruitment@jhu.edu. Applicants should also request that three letters of recommendation be sent electronically or to the address below:

Gerald W. Hart, Ph.D.
Faculty Search Committee Chair
C/O Ms. Angelina Hines
Department of Biological Chemistry
The Johns Hopkins University
School of Medicine
725 North Wolfe Street
Baltimore, MD 21205-2185

Equal Opportunity/Affirmative Action Employer.

UNIVERSITY OF MICHIGAN Interdisciplinary Cluster in Sustainable Food Systems

Three tenure-track faculty positions are currently available at the University of Michigan within a larger cluster focusing on sustainability of the food system. Positions are likely to be at the level of **ASSISTANT PROFESSOR**, although exceptional candidates at higher ranks will be considered. We are searching for individuals with interdisciplinary interests who take experimental or theoretical approaches to the problems of sustainable production and consumption of food. Each hire will have a university-year (nine-month) appointment in one of three tenure-granting units, the Department of Ecology and Evolutionary Biology (EEB), the School of Natural Resources and Environment (SNRE), or the School of Public Health (SPH). EEB seeks applicants working in ecological or evolutionary aspects of the food system from production to consumption. SNRE seeks natural scientists (including ecology, agroecology, agroforestry, hydrology, ecological modeling, spatial analysis, genetics, sustainability science, and related disciplines) working in any ecological aspect of sustainability in the food system with an international or domestic focus. SPH seeks applicants with expertise in sustainable food systems in relation to the environment, human health, and equity, including a focus on economic, racial and ethnic, urban and rural disparities in food distribution, relationships between food systems and environmental health, or their implications for under- or over-nutrition and related health outcomes. The SPH candidate will have a primary appointment in one of three departments—Environmental Health Science, Epidemiology, or Health Behavior and Health Education, depending on the candidate's interests and training. Teaching duties vary with curricular needs of the unit. The larger cluster includes positions in business and regional planning. Successful candidates will be expected to be active participants in the interdisciplinary activities of the Sustainable Food System Cluster.

To apply and for further information, see **website:** <http://sitemaker.umich.edu/sustainablefoodsystems>. Review of applications will begin on 1 October 2012. Women and minorities are encouraged to apply. The University is supportive of the needs of dual-career couples. The University of Michigan is an Equal Opportunity/Affirmative action Employer.

POSITIONS OPEN

POSTDOCTORAL FELLOW Bacterial Pathogenesis

Postdoctoral Fellow position is available immediately to join the collaborative research group of **Dr. Jeffrey D. Cirillo** studying tuberculosis pathogenesis. Selected individual will be primarily responsible for conducting independent research on mycobacterial pathogens and publication of results. Research will emphasize the molecular, cell biological, live animal molecular imaging and immunological characterization of virulence determinants in mycobacteria and their interactions with the host in mice and guinea pig virulence models. Ph.D. required and a record of productive experience in molecular biology of bacterial pathogens preferred. Interested applicants should apply online at **website:** <https://jobs.tamhsc.edu/postings/2118> and send curriculum vitae and names and addresses of three references postmarked by August 31, 2012 (or until a suitable candidate is found), to **Dr. Jeffrey D. Cirillo, Department of Microbial & Molecular Pathogenesis, Texas A&M Health Science Center, 8447 State Highway 47, 3107 Medical Research and Education Building., Bryan, TX 77807-3260. Fax: 979-436-0360; e-mail: jdcirillo9@gmail.com.** The Texas A&M Health Science Center is an Affirmative Action/Equal Opportunity Employer. Contact: **Dr. Cirillo, telephone: 979-436-0343** for additional information.

ASSISTANT PROFESSOR BIOCHEMISTRY

The California State University, East Bay (CSUEB) Department of Chemistry & Biochemistry invites applications for a tenure-track Assistant Professor position in Biochemistry (#12-13 CHEM-BIOCHEMISTRY-TT) for the 2013-2014 academic year. The successful candidate will have extensive training in biochemistry and a strong commitment to teaching. Applicants should be prepared to establish an externally funded research program in biochemistry for undergraduate and Master's students. Teaching responsibilities include biochemistry, advanced metabolism, and lower division chemistry lecture or laboratory courses. A Ph.D. is required; postdoctoral research, some teaching experience, and training in analytical biochemistry are preferred. Applicants should submit, via regular mail only, a cover letter specifying the position number, curriculum vitae, a one-page statement of teaching philosophy, a brief three-page statement of research plans, and arrange to have undergraduate and graduate transcripts and three letters of recommendation sent to: **Dr. Ann McPartland, Chair, Department of Chemistry and Biochemistry, California State University, East Bay, Hayward, CA 94542.** Review of applications begins September 14, 2012 and continues until the positions are filled. CSUEB, an Equal Opportunity Employer, is committed to the principles of diversity in employment.

ASSISTANT PROFESSOR

Department of Biology Bryn Mawr College

The Department of Biology at Bryn Mawr College invites applications for a full-time, beginning tenure-track Assistant Professor position in the area of Genomics to begin on July 1, 2013. We are searching for an individual who takes a genomics approach to biological problems using a combination of bioinformatic, statistical, computational, and biological methodologies, and who will thrive in an environment that combines teaching, research, and interdisciplinary collaboration. The successful candidate is expected to teach at all levels of the curriculum and establish an externally funded research program that provides rigorous collaborative research projects for undergraduates. Teaching responsibilities include courses in genomics and in the candidate's area of expertise, as well as involvement in the team-taught introductory biology sequence. A Ph.D. and at least one year of postdoctoral research experience are required. Submit a cover letter, curriculum vitae, research statement, and teaching philosophy that includes a short description of potential courses to be offered by October 5, 2012 to: **Genomics Search Committee, c/o Jodi Jacoby, Department of Biology, 101 North Merion Avenue, Bryn Mawr, PA 19010.** In addition, arrange to have three letters of recommendation sent to the same address. Bryn Mawr College is an Equal Opportunity Employer; minority candidates and women are especially encouraged to apply.

POSITIONS OPEN

SYSTEMS BIOLOGY University Of North Dakota

The Department of Biology at the University of North Dakota invites applications for a Tenure-track position in Systems Biology at the level of **ASSISTANT PROFESSOR**. The position is integral to our new undergraduate Major in Molecular and Integrative Biology and will strengthen the department's teaching and research mission in basic science. The individual will combine transcriptomic, metabolomic, and/or proteomic analysis with computational modeling exploring the role of gene regulatory networks or protein interactions in organismal function. The specific organism(s) or model system used is of less importance than the integrative nature of the analytical approach. Teaching duties will not exceed two courses per year during the first several years. A Ph.D. and postdoctoral experience are required. The successful candidates will demonstrate the potential to establish a productive and extramurally funded research program. The department offers graduate degrees through the Ph.D. and active training of graduate students is expected. The position will begin 16 August 2013. Review of applications will begin on or about October 1, 2012, and continue until the position is filled. Send curriculum vitae, three representative reprints, statement of teaching and research interests, and the names and contact information for at least three references as a single PDF to **Dr. Diane Darland at e-mail: diane.darland@und.edu.** Address mail contact to: **University of North Dakota, Department of Biology, 10 Cornell Street, Stop 9019, Grand Forks, ND 58202-9019.** For more information **website:** <http://www.und.edu/dept/biology/jobs.htm>.

The University of North Dakota is an Equal Opportunity/Affirmative Action Employer and we strongly encourage applications from women and underrepresented groups. Veteran's preference does not apply to this position.

MOLECULAR BIOLOGIST

Assistant Professor of Biology Amherst College

The Department of Biology at Amherst College seeks to fill a tenure-track position at the Assistant Professor level in molecular biology, to begin July 2013.

The successful candidate will mount an active research program that involves undergraduate students. Teaching duties include participation in lecture courses with laboratories in biochemistry, in molecular genetics, and in a team-taught introductory course in molecular and cellular biology. A completed Ph.D. is required and postdoctoral experience is expected.

Candidates should submit electronically a cover letter, curriculum vitae, separate research and teaching statements, and the names and e-mail addresses of three individuals to whom we may write to solicit recommendations. These materials should be submitted to **website:** <https://jobs.amherst.edu/view/opportunity/id/445>. Teaching statements should explicitly address the candidate's approaches to teaching biochemistry and molecular genetics. Review of applications will begin October 1, 2012, and will continue until the position has been filled.

Amherst College is a private undergraduate liberal arts college for men and women, with 1,700 students and a teaching faculty of 200. Located in the Connecticut River Valley of western Massachusetts, Amherst participates with Hampshire, Mount Holyoke and Smith Colleges and the University of Massachusetts in the Five College Consortium. Amherst College is an Equal Opportunity Employer and encourages women, persons of color, and persons with disabilities to apply.

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